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POLE BLIGHT OF WESTERN WHITE PINE: A NUMERICAL EVALUATION OF THE SYMPTOMS

THOMAS S. BUCHANAN, GEORGE M. HARVEY, AND D. S. WELCH[1]

(Accepted for publication August 15, 1950)

An unexplained decadence and premature death of young Western white pine (*Pinus monticola* Douglas) trees has been observed in northern Idaho, adjacent eastern Washington, and western Montana for several years, and more recently in the interior of British Columbia. When this disorder first began is not known, but it had attracted the attention of a few foresters as early as 1927 or 1928. Field observations were necessarily rather limited during World War II, but at the end of the war foresters were alarmed to find evidences of deterioration because of this unknown disorder in an estimated 70,000 acres of white pine forests. This estimate has since been revised to 100,000 acres, although systematic surveys have not yet been completed.

Various names have been proposed, but "pole blight" has come to be accepted (9). This is a logical name because the affected trees are primarily of pole size, 40 to 100 years of age. The first systematic study was a preliminary examination in 1938 of the root systems of Western white pine, made by the School of Forestry of the University of Idaho at the request of and in cooperation with the U. S. Forest Service and the Division of Forest Insect Investigations (3). A study to determine the cause of the trouble was begun in 1941 under a similar cooperative plan, but the war interfered (4). In 1947, the Forest, Wildlife, and Range Experiment Station of the University of Idaho undertook systematic studies of the characteristics and behavior of the disorder (8). These studies provided no conclusive findings but confirmed the serious threat to Western white pine and the urgent need of a full-scale program of research.

A rather precise description of the symptoms of pole blight is essential, to scout out the range and distribution of the disease, to follow its progress and development, and to provide possible clues as to its underlying cause. The Forest, Wildlife, and Range Experiment Station of the University of Idaho undertook as its highest priority assignment the responsibility for working out a description and severity classification capable of numerical expression. It was felt that a mere listing of the most frequently encoun-

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The work was done under University of Idaho Special Research Project No. 11, Dr. T. S. Buchanan, project leader. The authors gratefully acknowledge the valuable assistance of Dr. W. K. Ferrell, Dr. E. E. Hubert, H. P. Clark, F. G. Hawksworth, and T. F. Giles on field and office phases of this project.

tered symptoms, while of great value to the general forester, would be of little value for purposes of research. The results of this study are given here.

The numerical evaluation system described is based upon specific pole-blight symptoms and the change in growth pattern on affected trees accompanying increased severity. This index is adaptable for evaluating the condition of both individual trees and stands, resembling in these respects indices worked out for the littleleaf disease of shortleaf pine (6) and for blight of eastern white pine (7). The index is already being applied on 21 permanent plots, the data from which will aid in determining the average life expectancy of a tree or stand in a given stage of pole blight, so that salvage operations may be carried out ahead of serious loss.

FIELD WORK AS A BASIS FOR THE SEVERITY INDEX

From detailed studies in 1941 (4) and 1947 (8) and general observational data (10) it was determined that affected trees have yellowed foliage, stunted needle growth, thinned-out crown, and reduced leader growth, accompanied by pitch flow from lesions extending vertically up and down the trunk. This symptom description, however, gives only a general picture and is not capable of numerical expression. The field work basic to the numerical index was a detailed dissection in 1948 and 1949 of 58 Western white pine trees, from 36 to 89 years of age, selected throughout the main commercial range of the species in both healthy and diseased stands. The recorded data from 50 of these trees (eight were discarded because of blister-rust trunk cankers, mechanical injuries, or other vitiating factors) form the raw material from which the severity index was developed. The condition of the trees selected for dissection ranged from apparently healthy and vigorous in all respects through advancing stages of pole blight to near death. Thirty-one of the dissected trees were in the dominant crown class, 14 codominant, five intermediate, and none suppressed. The variation in number of trees in the respective crown classes was deliberate, the disorder being most common in the higher crown classes.

Field data. General information, such as location and environmental factors, was recorded for each tree selected and detailed measurements were then made. The following crown-portion designations were used for such measurements: Leader—the terminal shoot of the main stem. Upper crown—the five uppermost (youngest) whorls of lateral branches. Mid-crown—the mid-whorl of the living crown, plus the four whorls of lateral branches next above. Lower crown—whorls 3 to 7, inclusive, above the base of the living crown.

Measurements recorded.

1. Diameter breast-high, with diameter tape.
2. Width of live crown, with tape. The cardinal directions were marked on the bole and the tree was felled. The number of live whorls

was counted and a typical limb from each of the five whorls in the upper, mid-, and lower crown was tagged as to its whorl position in the live crown. The branches were then lopped and the stem cut into 10-ft. sections, beginning at 1-ft. stump height and continuing up the bole to the top. A 1-in. disc was sawed from the stem at each 10-ft. interval and marked as to its orientation and height in the tree.

3. Length of live crown, by direct measure.
4. Number of live whorls.
5. Total height of tree, by direct measure.
6. Length of internodes, by direct measurement back for 10 years on the leader; back from 1 to 5 years on the five branches from the upper crown; and back for 8 years on the five branches from the mid- and lower crowns, respectively (Fig. 1).

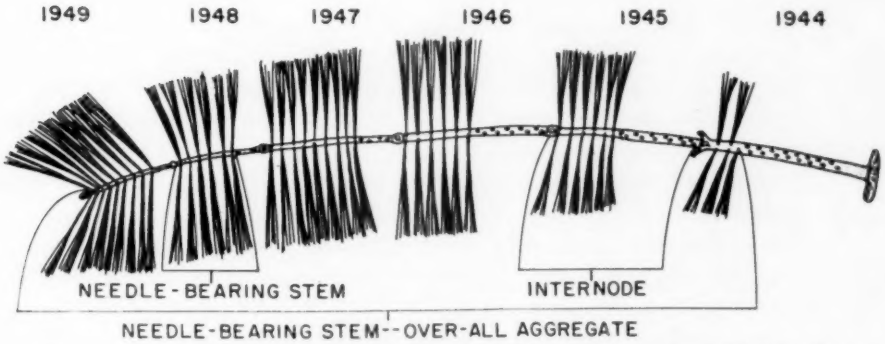

FIG. 1. Diagrammatic sketch of a Western white-pine branch, explaining the terminology employed in describing vigor.

7. Needle-bearing stem. Two types of measurement were made for this factor: 1) needle-bearing stem per internode, and 2) over-all aggregate needle-bearing stem (Fig. 1). These were taken for the leader and the same specimen branches as were measured for length of internodes.
8. Percentage needle retention: the percentage of needles remaining per internode, as estimated by comparing needles still attached with fascicular scars of fallen needle bundles. Leader, upper, mid-, and lower crown measurements.
9. Needle length and color. The needles were picked from the branches of each crown designation and further separated by years. A 25- to 30-gm. sample of needles of each age was collected into a small paper bag. At field headquarters, later in the day, ten fascicles were chosen at random from each bag and the average length of the ten entered against the branch number and year's internode on that branch. A larger sample was then used for matching the color of the needles with plates in the Dictionary of Color (5). The fifth

branch from the top only was used for upper crown, the mid-whorl only for mid-crown, the selected branch from whorl No. 7 from the base of the live crown for lower crown, and the leader as previously defined.

10. Moisture content of needles. The green weights of the needle collections were determined as quickly as possible after collection. The samples were later oven dried and their moisture content calculated on an oven-dry basis.
11. Moisture content of sapwood. A sapwood sample was cut from the north side of the discs removed from the bole of the tree. Green weights were taken at the same time as the needle weights, and the moisture content computed later on an oven-dry basis.
12. Stem analyses. A narrow wedge was cut from the south side of each of the discs removed from the stem. The annual growth at the various heights was measured back for 25 years, or for total age if under 25 years, to the closest 0.01 mm. The total age of the section was determined by total ring count, whether over or under 25 years.
13. Trunk or dissection tree diagrams. The outside and inside bark diameters of all the discs were measured with diameter tape before they were cut up for the sapwood and growth-measurement samples. The inside bark measurements were converted to circumference, in units and tenths of feet (the reverse side of the usual diameter tape). These values were then plotted to scale on cross-section paper (Fig. 2). In effect, the curved surface of the tree trunk was developed into a plane surface on paper. Each 10-ft. section of the trunk was then carefully inspected and all defects noted were drawn to scale on the stem diagram, with particular attention to location in reference to compass direction and height. All bark was then removed and areas of dead cambium and other defects were plotted to scale on the diagram (Fig. 2). Among the characteristic defects encountered, generally well up on the bole, were 1) narrow, elongate streaks where the underlying cambium had been dead for so long that the bark was beginning to crack and slough; 2) narrow flattened or sunken streaks where cambial activity had either ceased entirely or had been greatly retarded for a period of years (data obtained in 1947 showed similar streaks to have been dead or retarded as long as 9 years); and 3) exudation of resin from seemingly uninjured areas between nodes.

CONSTRUCTION OF THE INDEX

Early in the dissection work it became obvious that the dead cambial streaks on the trunk of the tree were the one symptom that could be considered specific for pole blight. The unexplained internodal resinosis and the flattened or sunken bark areas over dead cambium were also considered to be essentially specific symptoms. These dead cambial streaks, how-

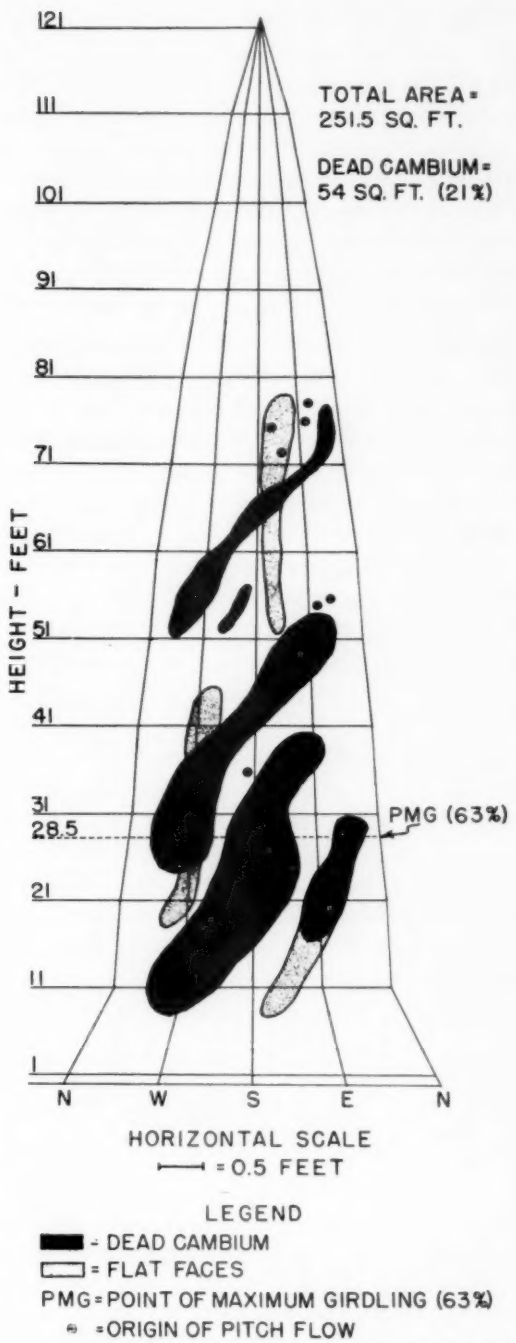

FIG. 2. Diagrammatic sketch of the surface development of the bole of a Western white pine with pole blight.

ever, were impractical symptoms for field use because of the time, labor, and wasted wood values involved in felling and dissecting a suspected tree in an effort to make positive diagnosis. For practical purposes, the determination of a numerical symptom description resolved itself into the selection of those above-ground, readily evaluated factors that had a high degree of correlation with the presence and abundance of dead cambial streaks.

All recorded growth measurements were first coded to avoid personal bias during statistical treatment, and then summarized. In the attempt to correlate variations in these factors with the dead cambial streaks it was soon apparent that there were still choices to be made. The dead cambial area had been planimetered on each tree diagram and calculated as a percentage of the total cambial area. The maximum girdling effect at any one height resulting from these dead streaks had also been calculated from the diagrams (Fig. 2). Trial plottings showed that using the percentage of maximum girdling as the independent variable yielded better correlations than the percentage of dead cambial area or any combination of these two cambial measurements.

All of the various growth measurements recorded in the field were then plotted over the percentage of maximum girdling effect. Those factors with only extremely low positive correlation, or none, were discarded by ocular inspection. The factors then remaining as items of high positive correlation were employed in the final symptom index (Table 1).

TABLE 1.—*Pole-blight severity index for Western white pine*

	Value		Value
External symptoms on trunk:		Needle length—upper crown:	
Normal	0.0	3 in. or more	0.0
Flat or dead faces	0.1	2½ in. or less	0.2
Unexplained resinosis	0.3	Needle color—upper one-fourth of crown:	
Flat or dead faces and unexplained resinosis	1.0	Normal	0.0
Length of internode of the leader, last complete year of growth:		Yellowish green	0.2
14 in. or more	0.0	Yellowish	0.6
13 to 9 in.	0.3	Dead	1.0
8 in. or less	0.6	Crown density—upper one-fourth of crown:	
Needle-bearing stem — overall aggregate of leader:		Normal	0.0
23 in. or more	0.0	Light	0.2
22 to 11 in.	0.2	Thin	0.4
10 in. or less	0.4		
Needle-bearing stem—average overall aggregate of upper crown:			
8 in. or more	0.0		
7 to 4 in.	0.2		
3 in. or less	0.4		

Since the classification employed in the Dictionary of Color did not lend itself to graphic analysis, the needle-color factor was carried into but four categories. These color variations, encountered in the approximate upper quarter of the crown, did correlate positively with the cambial girdling effect, however, and are retained as part of the final symptom picture.

Because no readily obtained measurement could be made of the crown density factor, it was evaluated only in terms of normal, light, and thin. The density of the upper one-fourth of the crown, estimated in these terms, showed positive correlation and hence was also retained in the final symptom index.

Plotting these variations in crown characteristics against each other showed some interdependence, particularly between upper-crown and leader values. Retention of all these characteristics has resulted in some seemingly unnecessary estimating for the observer. It was felt that the slight additional work involved was more than offset by usefulness as a system of checks and balances against human error. A poor estimate in one category will thus tend to be counteracted by a good estimate in another. There is no defense, however, against the possibility of an observer's making a poor estimate in all of the characteristics employed.

In assigning numerical values to the various crown-symptom factors, the respective curves were simply broken at critical points to arrive at the categories in table 1. All crown symptoms are not given equal weight; those factors having the highest correlation with the percentage of cambial girdling effect were given the greatest, and those with poorer correlation, the least.

Numerical values for external symptoms on the trunk were also assigned in relation to their dependability. Unexplained resinosis was about three times as dependable in indicating dead cambium beneath as were flat faces detectable from the ground, so was given three times as much weight. When flat faces and resinosis were both apparent on the bole, dead cambium was consistently found to be present; hence these factors occurring in combination were given more weight than their added individual reliabilities would indicate. In practice, the factors listed under external symptoms on the trunk are used to determine whether or not the tree actually has pole blight, and the remaining factors merely measure the relative severity of attack.

USING THE INDEX

To employ the index, the tree is examined for the variables noted in table 1 and the proper classes are checked. A simple summation of the checked values gives the numerical index for the tree: 0.0–0.5, normal, healthy tree; 0.6–2.0, probable early pole blight; 2.1–3.0, definite pole blight; 3.1–3.6, severe pole blight; 4.0, dead tree. Totaling individual indices for all trees on a plot and dividing this total by the number of trees

examined gives a unit figure expressing the condition of the plot, and hence of a given stand, as a whole.

As an example, assume the field application of these results to a hypothetical tree. Careful examination of the bole at close range shows copious resinosis originating between nodes about 20 to 30 ft. from the ground, as well as a noticeably flattened streak a few inches wide spiralling around the tree from 15 ft. to 40 ft. above the ground. Since the resinosis cannot be explained on the basis of any known causal agent and since the flattened streak is not associated with root crotches, a value of 1.0 (Table 1) is assigned to these factors.

Examining the leader with the aid of binoculars, the length of the last internode is estimated to be 4 in., giving a numerical value of 0.6; the over-all aggregate length of needle-bearing stem on the leader is estimated at 12 in., numerical value 0.2; and the upper crown, an average aggregate of 5 in. of needle-bearing stem, numerical value again 0.2. Needle length in the upper crown is determined, however, as being a normal 3 in. plus, hence the numerical value 0.0 is assigned. Needle color in the upper crown is definitely off normal, a yellowish green, numerical values 0.2. Density of the upper crown, being also subnormal, is rated as "light," numerical value 0.2. The total numerical value is 2.2, indicating the tree has definite pole blight. Reexamination of this same tree one year later will not only show whether or not its condition has changed, but will provide a concrete measure of that change.

DISCUSSION

Since, obviously, many causal agencies could induce crown symptoms similar to those evaluated in table 1, the results must be rather carefully interpreted. The cause of pole blight has not been determined, but the presence on the main bole of dead cambial streaks is considered to be a specific symptom. Root rots and other root defects may also cause dead cambial streaks, but these are typically associated directly with the defective root, extending up the bole a variable distance above that root. The dead cambial streaks considered typical of pole blight commonly begin 6 to 10 ft. above the ground, extending in many cases well up into the living crown of the tree. The dead wood behind these streaks becomes blue-stained very quickly.

The dead cambial areas generally spiral up the tree to the right (Fig. 2). This condition is more apparent than real, the streaks actually following the grain of the wood. All trees dissected had such spiralling. Dead cambial streaks occurred at random on all sides of the bole.

Measurements of radial growth on the stems of pole-blighted trees failed to show a positive correlation with the severity of attack. It is true that pole blight retards radial growth, but the effect is so variable in degree and time of occurrence that it cannot be used as a diagnostic feature. Even

more confusing is the tremendous variation in radial growth pattern encountered at different heights along the bole, even on the same side of the tree.

Surprisingly enough, no significant differences were found in the moisture content of the sapwood of trees in varying stages of decline. Similarly, the moisture content of the foliage, sampled from various portions of the crowns, showed no correlation with the severity of pole blight.

The numerical system devised (Table 1) does, however, serve satisfactorily to identify pole-blighted trees and to evaluate the severity of attack. Although the chronological sequence of symptom appearance has not yet been determined, nor is detection of the very earliest symptoms yet definitely possible, the tabular data can be used to classify trees in known pole-blight centers. In making positive identification of newly discovered areas of suspected pole blight, however, the final positive diagnosis is still dependent upon felling and removing the bark from the bole of the suspected tree and the finding of dead cambial streaks thereon.

SUMMARY

Streaks of dead cambium, generally a few inches wide and several feet long, occurring on the main bole were the only symptom found upon detailed dissection of 50 trees that could be considered specific for pole blight. Since this symptom cannot be detected except by felling and removing the bark from the tree, it is not a practical one for field use. Unexplained resinosis and flat faces on the bole were, however, reliable indicators of dead cambium beneath. Still other readily measured crown symptoms correlated positively with the girdling effect of these dead cambial streaks, and a reliable symptom pattern for field use has been developed on these bases.

The following observable characteristics were reliable expressions of the severity of pole blight and were utilized in developing the symptom picture: 1) Unexplained internodal resinosis on the main bole. 2) Dead areas on the main bole, or flattened areas not directly associated with root crotches. 3) Length of the last complete year's internode on the leader. 4) Over-all aggregate length of needle-bearing stem on the leader and upper crown branches. 5) Length and color of needles in the upper crown. 6) Density of the upper crown.

Numerical values have been assigned to variations in these factors in such a way that an apparently healthy tree has a score of 0.0 and a dead tree a score of 4.0. Trees in various stages of pole blight will score between those extremes.

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TOBACCO RING-SPOT VIRUS: THE CAUSE OF EGGPLANT YELLOWS[1]

W. D. VALLEAU

(Accepted for publication October 15, 1950)

Eggplant yellows has caused considerable injury to eggplants year after year in the Rio Grande Valley of Texas. The same virus causes mosaic of cantaloupes in the Valley (1). Dr. Ivanoff, in correspondence, stated that he had induced the disease on eggplants by inoculating with juice from mottled cantaloupe leaves, but was not certain whether the virus was that of cucumber mosaic (Porter's), or ring spot, or both. In his last publication (1) on the subject he concluded that "the virus appears to be closely related to the cucumber group of viruses." Living material of affected eggplant was obtained from Dr. Ivanoff for a comparative study of the virus with tobacco viruses.

The present paper reports the results of inoculations 1) on burley tobacco with the eggplant material sent by Ivanoff, and 2) on eggplant with ring-spot virus from tobacco; and offers an explanation of the control of yellows of eggplant with sulfur dust.

INOCULATION OF TOBACCO WITH EGGPLANT YELLOWS

Three mosaic-resistant burley tobacco plants (N factor from *Nicotiana glutinosa*) were inoculated, December 3, 1941, with crushed leaves from eggplants affected by yellows. By December 16, chlorotic spots had developed on inoculated areas, and necrotic rings were beginning to develop. Chlorotic areas were removed from each tobacco plant, crushed in disodium phosphate solution, and inoculated to three upper leaves of the respective plants. The potted plants were then transplanted to a ground bench. Within 6 days, 103 chlorotic spots, often with a necrotic center or a small necrotic ring, had developed from the second inoculations. Later they developed necrotic rings typical of ring spot. On January 3, 1942, the plants were growing rapidly, but systemic infections had not developed and the plants were cut back to the upper inoculated leaf to determine whether ring patterns would develop in the lateral shoots. Typical ring-spot patterns developed in the new leaves of only one plant. However, when the lateral shoots on the other plants bloomed, only a few seeds set because of nearly complete pollen sterility. As partial pollen sterility is a characteristic symptom of ring spot (5), this indicated that the plants were systemically invaded, probably as a result of invasion of the lateral bud before it commenced growth (so-called recovery). One plant later sent out a lateral

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

shoot from the ground line. When the shoot was about 3 ft. tall, necrotic ring patterns typical of tobacco ring spot developed on all 16 leaves of this shoot as a result of delayed invasion.

These inoculations, while limited in number, indicated that the tobacco ring-spot virus was present in eggplants with yellows and was probably the cause of yellows. This conclusion is based on (a) the development of symptoms, at the points of inoculation, that were typical of the tobacco ring-spot virus; (b) the development of systemic symptoms consisting of chlorotic and necrotic ring patterns typical of some strains of the tobacco ring-spot virus; (c) so-called recovery of plants from the ring-spot stage of the disease; and (d) pollen sterility.

TOBACCO RING-SPOT VIRUS IN EGGPLANT[2]

Wingard reported infection of eggplant with the tobacco ring-spot virus but did not transfer the virus back to tobacco. Neither did he describe the disease produced in eggplant (6). E. M. Johnson failed to produce symptoms in eggplant inoculated with tobacco ring-spot virus, and failed in attempts to transfer the virus from inoculated plants back to tobacco (3).

In the present study, cotyledons of 28 eggplant seedlings, of an unknown variety, were rubbed with inoculum of a typical strain of ring-spot virus, on November 2, 1948. Symptoms did not develop, and on November 24 the plants were reinoculated, using carborundum, with virus-containing tobacco leaves crushed in disodium phosphate. No symptoms were evident December 1, when tobacco leaves inoculated at the same time had heavy infection. The eggplants were again inoculated. On December 13 they had no local lesions and were set in the ground bench. On December 16 a piece of noninoculated eggplant leaf was removed from each of six plants, crushed together in M/10 disodium phosphate, and inoculated to a tobacco plant. Another set of six leaves from six other plants was treated likewise and inoculated to a second tobacco plant. Both inoculations resulted in numerous necrotic spots on the inoculated leaves, indicating that the ring-spot virus was present in noninoculated leaves of at least some of the symptomless eggplants. Subsequently numerous transfers of the ring-spot virus were made to tobacco from many individual symptomless eggplants, both with expressed juice and by grafting eggplant on tobacco. None of the eggplants growing in the ground bench in the greenhouse from December until late spring had signs of eggplant yellows, although the plants were systemically infected with the ring-spot virus.

In March 1949, eggplants in the four-leaf stage were inoculated with another strain of the ring-spot virus, a strain that caused yellowing of dark tobacco in the field. The virus was obtained from yellow seedlings grown from seed of the yellowed tobacco plant. Eggplants inoculated with this strain of the virus were set in a ground bench, but no yellows developed during the ensuing months.

[2] The able assistance of Mr. T. C. Chu in this study is gratefully acknowledged.

EFFECT OF RING-SPOT VIRUS ON POLLEN DEVELOPMENT IN EGGPLANT

On February 25, 1949, pollen from 10 eggplants infected with the non-yellowing strain of tobacco ring-spot virus was examined. In some instances the anthers appeared not to have opened. In all cases pollen was scarce and could be shaken or scraped from the anther with difficulty. The pollen was mounted in 20 per cent lactic acid, which quickly swells normal grains but does not burst them. Of 16,964 grains examined, 40.4 per cent were abortive. The percentage of abortive pollen ranged from 22.6 to 71.1 for different anthers. On May 25, pollen from five plants inoculated with the yellowing strain of the virus was examined, and of 1,095 grains, 17.9 per cent were abortive. Of 852 pollen grains from four healthy plants, 3.2 per cent were abortive.

In tobacco, different strains of the virus cause different degrees of pollen sterility (4, 5). What the effect of infection in the field on fruitfulness of eggplants would be is a matter of speculation. Eggplants affected by one or the other of the two virus strains set some fruit without hand pollination. It seems probable that in a crop of eggplants in which a considerable number of plants are affected by yellows, partial pollen sterility will tend to decrease fruit set.

A POSSIBLE VECTOR OF RING-SPOT VIRUS

In Texas (2) it has been demonstrated that eggplant yellows in the plant bed and in the field can be controlled by dusting with sulfur. In view of the fact that eggplant yellows appears to be caused by the tobacco ring-spot virus, this result opens up interesting possibilities as to the use of sulfur as a tool in determining the vector, as well as possibilities of controlling tobacco ring spot, particularly in seed crops.

While the vector has not been demonstrated, the following circumstantial evidence suggests that it may be *Thrips tabaci* L. This insect appears in large numbers on tobacco soon after setting and remains until tobacco commences rapid growth, when it largely disappears. Simultaneously ring spots appear on tobacco leaves, usually as single local lesions on occasional plants. According to records taken in the summer of 1949, all ring-spot infections on tobacco originate within a month, and probably sooner, after setting, although systemic invasion may not occur until or after plants begin to elongate.

If *Thrips tabaci* is the vector, only a small proportion of the insects cause infection: in a field of 1,500 plants, all heavily infested with the insects, only 6 per cent of the plants became visibly infected, and of these 25.5 per cent did not develop systemic infection. The transfer of hundreds of thrips from field tobacco to small plants in the greenhouse failed to furnish proof that they were able to transmit the virus.

It may be necessary to find some highly susceptible host in which symp-

toms, other than ring spots, are prominent before progress can be made in determining the insect vector.

It has been observed during two seasons that as soon as a plant is dusted with sulfur the thrips become very active and soon leave the plant. The next day they are found in very small numbers, perhaps one or two per plant, on areas that escaped dust.

DISCUSSION AND CONCLUSIONS

Transmission studies proved that the yellowed eggplant contained tobacco ring-spot virus, which would produce typical ring-spot symptoms and cause pollen sterility in burley tobacco.

Although yellowing did not develop in greenhouse eggplants inoculated with two strains of tobacco ring-spot virus, the eggplants were systemically infected and the virus could be recovered from crushed leaf tissues. It is possible that light conditions during the experiments were responsible for lack of yellows symptoms.

Lack of ring patterns in the eggplants invaded by the tobacco ring-spot virus suggests that eggplant is possibly more susceptible to the virus than tobacco and numerous other plants in which chlorotic and necrotic rings develop; but the disease in eggplant is very similar to the disease in tobacco, potato, and *Solanum carolinense* in the chronic, or so-called recovered, stage when ring patterns are no longer produced but certain strains of the virus cause a typical yellows disease.

Partial pollen sterility occurs in eggplant invaded by the ring-spot virus just as it does in tobacco. Pollen sterility might reduce the fruitfulness of eggplants as it does tobacco.

While the insect vector of the ring-spot virus is not known, sulfur controls eggplant yellows and it has the immediate effect of driving *Thrips tabaci* from tobacco plants. *Thrips tabaci* are abundant on tobacco during the time infection with ring spot takes place.

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FURTHER STUDIES ON THE PROPERTIES OF POTATO YELLOW-DWARF VIRUS

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Because potato yellow-dwarf virus (*Aureogenus vastans* (Holmes) Black) is the only one of the leafhopper-borne viruses known to produce primary lesions on inoculated leaves, it seems to provide a logical point of attack on this little-understood group. A few of the properties of potato yellow-dwarf virus in the juice of Indian tobacco plants (*Nicotiana rustica* L.) were reported earlier (2). The present paper reports work, most of which was carried out at the Rockefeller Institute for Medical Research, to further elucidate the properties of this virus. A concise account of some of the experiments has already appeared (4).

MATERIALS AND METHODS

The *vulgare* (3) variety of potato yellow-dwarf virus was used.

Nicotiana rustica plants were employed both as sources of virus and as test plants. In most experiments the activities of two virus preparations, treated differently in one respect, were compared by inoculations on opposite half-leaves of *N. rustica*. Often they were compared at different dilutions, all of which were made in 0.1 M neutral potassium phosphate buffer. All pH determinations were made by means of a glass electrode.

On each plant only the two leaves (in a few experiments, only a single leaf) estimated to be most susceptible were inoculated. An attempt was made to describe these leaves in an earlier paper (2), but so many factors affect the growth of the plant and its susceptibility that experience in inoculating all the leaves on plants over a range of ages is probably the best way of learning which ones produce readily counted primary lesions. The shoot above the selected leaves was always broken out at the time of inoculation and, until after the lesions were counted, the axillary shoots were broken out shortly after they appeared. Leaves to be inoculated were dusted with carborundum No. 320 from a pepper shaker, with a small cheesecloth bag tied around the neck to aid in the dispersal of the carborundum. The leaf surface was rubbed three times rather firmly with a cheesecloth pad soaked in inoculum.

Because of the great variability in susceptibility from leaf to leaf and plant to plant, only two samples were compared on the leaves of any one plant. For example, to compare samples 1 and 2, sample 1 would be applied to the left halves of both selected leaves of plants 1 to 8; sample 2 to the right halves. On plants 9 to 16 the positions would be reversed. The comparison of 1 and 2 on opposite half-leaves was fairly reliable, but comparisons of such data with other data—for example, data on other dilutions

of samples 1 and 2 inoculated on other leaves in the same experiment—were less reliable. For this reason, a footnote to each table indicates which data are from opposite halves of the same leaves.

In spite of the fact that extra light was provided the plants in the winter, and shade during the summer, some experiments yielded poor or entirely negative results and are not reported. Observations during several years suggested that in winter when days were short and light intensity low, the virus content of diseased *N. rustica* plants was lower than in summer. On the other hand, the plants appeared to be more susceptible to inoculation in winter than in summer. In the greenhouse in early summer, lesions may begin to appear in 6 days; under poor conditions in late summer they may not appear until 2 weeks have elapsed.

STUDIES ON VIRUS IN JUICE OF NICOTIANA RUSTICA PLANTS

In some experiments potato yellow-dwarf virus was demonstrable in juice of *Nicotiana rustica* plants stored at room temperature (about 25° C.) for 2½ hr. after extraction but not at later intervals; in other experiments, it was detected after 12 hr. but not 24 hr. (2). For work on purification, it was desirable to increase the longevity *in vitro*.

Longevity in neutralized juice at room temperature. Fresh juice of *Nicotiana rustica* leaves has a pH of about 5.25 to 5.45. It can be neutralized by dissolving 4 gm. of Na_2HPO_4 in each 100 ml. of juice. In two experiments samples of neutralized and unneutralized juice were stored for 5 min. and for 6 hr. at room temperature. At the end of the storage periods, equal amounts of Na_2HPO_4 were added to the samples of unneutralized juice, all samples were diluted 1:10 in buffer and contrasting solutions compared by inoculating each on 12 opposite half-leaves of *N. rustica* plants. In both experiments numerous lesions were produced by both neutralized and unneutralized juice stored for 5 min., but none when they were stored for 6 hr. Thus, no beneficial action of neutralization at room temperature was demonstrated.

Longevity in unneutralized juice at low temperatures. The preservative effect of low temperatures on plant viruses *in vitro* has been emphasized in many studies. To test the effect of low-temperature storage on potato yellow-dwarf virus, samples of juice (pH 5.4) from diseased plants were stored at 25° C. and at either 5° or 0° C. for various intervals, and then tested for virus activity. In the first experiment, the 25°, 2-hr. sample produced 313 lesions on 16 half-leaves; the corresponding 5° sample produced 1,644 lesions on the opposite half-leaves. Corresponding samples stored for 4 hr. produced 135 and 2,000 lesions, respectively. In a second experiment, a 25°, 12-hr. sample produced 0 lesions and a corresponding 0° sample 4,957; the 24-hr. samples, 0 and 331 lesions, respectively. The low temperatures had a marked preservative value.

Longevity in neutralized juice at low temperatures. To investigate the possibility that neutralization might have a beneficial effect at 0° C., samples

of neutralized and unneutralized juice from diseased *N. rustica* plants were stored at this temperature and tested for activity after 2 days and again after 7 days. Neutralization was obtained by adding 0.65 ml. of a solution containing 0.5 gm. K_2HPO_4 per ml. to each 10 ml. of juice. The phosphate was added to the neutralized samples at the beginning of the storage period and to the unneutralized samples at the end of the period. The undiluted solutions were each tested on 12 opposite half-leaves of *N. rustica*.

In one experiment the 2-day neutralized sample produced 887 lesions; the corresponding solution, stored unneutralized, 694. After 7 days' storage, the corresponding data were 1,155 and 38, respectively. A second experiment gave similar results. These data are in harmony with later experiments on the effect of H-ion concentration on partially purified virus.

Effect of freezing diseased leaves on virus content of expressed juice. Because of the instability of potato yellow-dwarf virus, it was thought that freezing diseased leaves before expressing their virus-containing juice might inactivate the virus in the leaves. This method was compared with that of expressing juice from leaves cooled to a low temperature without freezing. Small pieces of diseased *N. rustica* leaves, about 1 in. square or less, were thoroughly mixed and then separated into two lots. K_2HPO_4 was added to each lot at the rate of 4 gm. of the salt to 100 gm. of leaves. One lot was frozen for 45 min. at $-14°$ C.; the other cooled to 7° C. Each sample was ground with a mortar and pestle and the juice squeezed out through cheesecloth. The two samples were then compared on opposite half-leaves of *N. rustica* at different dilutions. Although the first experiment (4) indicated some detrimental effect of the freezing, two later experiments failed to show any consistent difference in the activity of the solutions obtained by the two methods. However, in practice poor virus activity sometimes resulted when large quantities of leaves were frozen and thawed before the juice was squeezed out. This may have been caused by local overheating in an endeavor to secure thawing in a reasonable length of time. Because of this difficulty and because extraction from cooled fresh leaves yielded as much virus, the latter technique was followed in the remainder of the experiments.

Later, virus partially purified by three ultracentrifugations was frozen at $-14°$ C. for 24 hr. After thawing, the solution was compared with another aliquot that had been stored without freezing at 0° C. Each sample at a dilution of 10^{-3} was inoculated on 16 opposite half-leaves. The frozen sample produced 664 lesions, the other 1627, indicating that considerable virus had been destroyed by the freezing.

Longevity of the virus in frozen leaves. In two tests undiluted juice from leaves kept at -13 to $-15°$ C. for 4 weeks was applied to 10 half-leaves on five *N. rustica* plants. The opposite half-leaves were left uninoculated as a control. Undiluted juice from fresh diseased leaves was applied to 10 half-leaves on five other *N. rustica* plants, the opposite half-leaves again being left uninoculated. None of the uninoculated half-leaves developed lesions. In one experiment, juice from leaves frozen for 4 weeks

produced 977 lesions, that from fresh leaves an estimated 5,800. Similar results were obtained in a second experiment. Apparently considerable inactivation of the virus occurred during such storage. Diseased leaves kept frozen at – 13 to – 15° C. in an atmosphere saturated with water vapor to prevent desiccation failed to produce infection when used as inoculum after 7 and 8 months storage. In spite of these results, it may well be that the virus in diseased leaves could be stored for long periods by quick freezing and storage at much lower temperatures.

Different leaves as a source of virus. Leaves on diseased *N. rustica* plants may be roughly classified into three groups: 1) inoculated leaves covered with primary lesions, near the base of the plant; 2) leaves first to be systemically invaded, and having acute yellow mottling; 3) chronically affected leaves at the top of the plant, with veinclearing, rugosity, dwarfing, and light green mottling, but much less yellowing than in acutely affected leaves. To determine whether leaves in all three categories were satisfactory as sources of virus or whether the virus was concentrated to a considerable extent in the acutely affected leaves, samples of juice from inoculated leaves and from chronically affected leaves were compared at three different dilutions with juice from acutely affected leaves on the same plants. The results (Table 1) indicate clearly that both the inoculated and the

TABLE 1.—*Different parts of Nicotiana rustica plants as sources of virus*

Experiment number, and source of virus	Number of lesions on eight half-leaves of *N. rustica* at dilutions of[a]		
	10^{-1}	10^{-2}	10^{-3}
1—Acutely affected leaves	4576	1645	54
Inoculated leaves	3877	727	19
2—Acutely affected leaves	*	845	83
Chronically affected leaves	*	765	88
3—Acutely affected leaves	*	1451	169
Chronically affected leaves	*	2973	570
4—Stems	2235	1158	149
Leaves	481	568	17
5—Stems	1581	179	10
Leaves	374	38	1
6—Stems	*	1196	397
Leaves	*	1816	306
7—Stems	1297	135	12
Leaves	1544	254	14
8—Stems	3106	525	26
Leaves	3090	257	11

[a] In each experiment, opposite halves of the same leaves furnished data for the two kinds of material at a given dilution.

* Lesions were too numerous to count.

chronically affected leaves may contain large amounts of the virus, and that all the leaves may usefully serve as a source.

Stems as sources of virus. *Nicotiana rustica* stems affected with potato yellow-dwarf virus have a mottled surface. Juice from such stems does not contain as much chlorophyll as does juice from affected leaves. A num-

ber of comparisons were made at different dilutions, between the infectivity of juice from stems and that from leaves (Table 1). In some tests, the stems yielded more infectious solutions than the leaves; in others the opposite was true. It is apparent, however, that the stems are a good source of virus.

SEDIMENTATION OF THE VIRUS

Attempts were made to purify the potato yellow-dwarf virus by means of the techniques developed by Stanley and Wyckoff (5) and others for the purification of tobacco-mosaic virus. The diseased leaves used in purification experiments were first cooled to about 0° C. and the virus maintained at about that temperature in all subsequent treatments. The leaves were then ground, 4 gm. of Na_2HPO_4 were mixed with every 100 gm. of pulp, and the juice was squeezed through cheesecloth and filtered through medium grade Hy Flo Super Cel. The celite removed most of the chlorophyll. The virus-containing solutions were spun in an angle-head centrifuge running at 550 r.p.s. and producing a mean gravitational field of about 50,000 × gravity.

The first solutions were ultracentrifuged for about 1½ hr. Each centrifuge tube contained 14 ml. of solution. The upper 9 ml. of the supernatant were then pipetted off and the next 5 ml. of liquid discarded. The supernatant and resuspended sediment were applied to opposite half-leaves of *N. rustica* at various dilutions. Because the pelleted sediment was resuspended in half of the original volume, or 7 ml. of neutral phosphate buffer, any result in which the two samples (supernatant and pellet) proved equally infectious would indicate that the sediment contained roughly half as much virus as the supernatant. After evidence was obtained that the virus could

TABLE 2.—*Sedimentation of potato yellow-dwarf virus in a mean field approximately 50,000 × gravity*

Time centrifuged, and experiment number		Number of lesions produced in eight half-leaves of *Nicotiana rustica* by the supernatant (S) and the resuspended pellet (P). Pellets were resuspended in half the original volume. S and P were compared at dilutions of[a]							
		10^{0}		10^{-1}		10^{-2}		10^{-3}	
		S	P	S	P	S	P	S	P
84 min.:	1	182	*	16	3580	2	1812	.....	.....
	2	114	*	13	3660	3	1553	.....	.....
30 min.:	3	61	4104	19	3022	2	1047	.....	.....
	4	381	5859	46	3504	1	1098	.....	.....
20 min.:	5	.....	.....	3	2158	0	321	0	14
	6	.....	.....	44	931	15	138	1	5
10 min.:	7	.....	.....	44	547	4	46	0	2
	8	.....	.....	23	1147	4	110	0	6
1 min.:	9	.....	.....	145	799	28	79	4	7
	10	.....	.....	522	872	45	125	1	5

[a] At each dilution within an experiment, the supernatant (S) and the resuspended pellet (P) were on opposite halves of the same leaves.

* Lesions were too numerous to count.

..... indicates not tested.

be successfully sedimented by ultracentrifugation for 84 min., shorter intervals were tested. The data (Table 2) indicate that 90 per cent or more of the virus was sedimented by ultracentrifugation for 10 min. Even centrifugation at 550 r.p.s. for 1 min. deposited roughly half of the virus in the pellet. The time required for the ultracentrifuge to attain a speed of 550 r.p.s. and to stop, 9 min. in each case, is not included in the time intervals given in the table. Potato yellow-dwarf virus sedimented much more rapidly than tobacco mosaic virus was known to do, suggesting that it was the largest plant virus studied in the ultracentrifuge up to the time of these experiments.

In several experiments, alternate ultracentrifugation and celite filtration, or alternate high- and low-speed centrifugation, were repeated one to three times in an effort to rid the preparations of chlorophyll completely, but the best preparations remained light green or light yellow. Moreover, similar treatment of juice from healthy plants yielded preparations of similar appearance which were noninfective. It was obvious that although the virus had been concentrated, it was still far from pure.

EFFECT OF HYDROGEN-ION CONCENTRATION

To determine the effect of hydrogen-ion concentration, a suspension of the virus was prepared from neutralized juice that had been filtered through celite. The virus was concentrated by means of two ultracentrifugations, the sedimented virus being resuspended each time in distilled water. After each ultracentrifugation, the resuspended virus was cleared of coarser material by means of a Swedish angle centrifuge. A small volume of this concentrate (0.2 ml.) was added to 9.8 ml. of a complex buffer solution of the desired pH, prepared according to the method of Best and Samuel (1). The virus was stored at 0° C. for 1 hr. or 24 hr., as desired; then 1 ml. of the mixture of virus and complex buffer was added to 9 ml. of neutral 0.1 M phosphate buffer, and this solution and a control were applied to opposite halves of 16 *N. rustica* leaves. The control in each case consisted of virus treated in the same manner except that it had been placed in a complex buffer solution of pH 7.0. The pH of the control after storage for 24 hr. at 0° C. and after readjustment was in each case 7.0. Determinations of pH were made on aliquots of the different solutions. The lesion counts on the various samples, and the percentage relationship between test sample and control, were determined (Table 3). The extreme variation in lesion count on the controls was due largely to the contamination of some leaves with lesions of tobacco-mosaic virus, so that all or part of the contaminated half-leaf, and a corresponding area of the opposite half-leaf, were discarded. Although this reduced the number of half-leaf comparisons in some lots, it is believed that the experiment as a whole gives a fairly reliable picture. The data for the 1-hr. and 24-hr. treatments are in good accord. Another source of error in the experiment was the marked shift in pH in two of the alkaline samples during treatment (Table 3).

In a repetition of part of the experiment, virus stored at pH 4.0 and 0° C. for 1 hr. yielded about 5 per cent of the number of lesions given by the control. There was also no evidence of reduction in infectivity up to pH 8.7 for 1 hr. at 0° C. However, it is apparent that inactivation of the virus was rapid at and below pH 5.0 and at and above pH 10.0. The virus was stable over a wider range above neutrality than below.

TABLE 3.—*Effect of hydrogen-ion concentration on stability of potato yellow-dwarf virus partially purified by two ultracentrifugations*[a]

pH of buffer before addition of virus	pH of buffer plus virus after 24 hr. at 0° C.	pH after readjustment after 24 hr. at 0° C.	Treatment 1 hr., 0° C.			Treatment 24 hr., 0° C.		
			Number of lesions on 16 half-leaves		Test sample as percentage of control	Number of lesions on 16 half-leaves		Test sample as percentage of control
			Test sample	Control		Test sample	Control	
2.00	2.03	6.95	0	1636	0.0	0	2248	0.0
3.00	3.02	7.00	3	1867	0.2	0	2995	0.0
4.00	4.00	7.04	0	1316	0.0	0	3186	0.0
5.02	4.98	7.03	329	781	42.1	24	2373	1.0
5.99	5.98	7.04	1442	1011	142.6	2395	2195	109.1
7.97	7.98	7.06	2015	2298	87.7	1455	2176	66.9
9.00	8.90	7.07	2378	2777	85.6	1997	3087	64.7
10.00	9.62	7.09	2471	2996	82.5	405	1077	37.6
11.00	10.10	7.10	2808	4235	66.3	550	3260	16.9
12.06	11.93	7.28	0	4919	0.0	0	2913	0.0

[a] For each pH value the test samples and the controls within a treatment were on opposite halves of the same leaves.

BERKEFELD FILTRATION OF ULTRACENTRIFUGED VIRUS

Virus partially purified by the ultracentrifugation technique was still filterable through Berkefeld N and W filters. In one experiment, partially purified virus in 0.1 M neutral phosphate buffer was passed through a Berkefeld N filter. No bacteria were added to those that might be expected to occur as natural contaminants of the preparation before filtration. Each of two test tubes of nutrient dextrose broth receiving 1 ml. of the filtrate remained sterile. The Berkefeld N filtrate at a dilution of 10^{-2} produced 137 lesions on eight half-leaves. In a second experiment, a mixture of 1 ml. of the partially purified virus, 1 ml. of a 1-day-old broth culture of *Agrobacterium radiobacter* (Beijerinck & van Delden) Conn., and 38 ml. of 0.1 M neutral phosphate buffer was passed through a Berkefeld W filter. When 1 ml. of the filtrate was added to each of two test tubes of broth, they remained sterile. The filtrate diluted to 10^{-1} produced 45 lesions on eight half-leaves, the unfiltered solution producing 49.

STABILITY OF PARTIALLY PURIFIED VIRUS

Potato yellow-dwarf virus partially purified by two ultracentrifugations was stored in 0.1 M neutral phosphate buffer at 0° C. and tested for activity after 2 weeks and again after 4 weeks. In both experiments it was

found that the virus was still active after 4 weeks' storage at 0° C., in one case at a dilution of 10^{-2} and in the other at a dilution of 10^{-3}. It was apparent, however, that considerable activity was lost during the 4 weeks.

SUMMARY

Although active potato yellow-dwarf virus could not be demonstrated in untreated juice of diseased *Nicotiana rustica* plants after a period varying from 2½ to 12 hr. at room temperature (about 25° C.), juice neutralized and stored at 0° C. for a week was still very infectious.

There was no important difference in the virus content of juice obtained from cooled fresh leaves and that obtained from frozen leaves. Although the virus survived in frozen leaves at −14° C. for 1 month, it had disappeared after 7 months of such storage.

Inoculated leaves of *N. rustica* and leaves in both the acute and chronic stages of the disease were suitable sources of virus. The stems of diseased plants were also a rich source of virus.

In a mean gravitational field of 50,000 × gravity, 90 per cent or more of the virus was sedimented to the bottom of the tubes in 10 min. plus the time it took to attain speed and to stop the machine. The virus sedimented more rapidly than tobacco-mosaic virus. It probably is the largest plant virus studied in the ultracentrifuge up to the present time.

Preparations of potato yellow-dwarf virus were obtained by one to three ultracentrifugations. In none of these were the plant pigments completely removed.

The stability of partially purified virus towards hydrogen-ion concentration was studied. The virus was most stable between pH 6.0 and 9.0. It was stable over a greater pH range on the alkaline side of neutrality than it was on the acid side.

Ultracentrifuged virus was readily filterable through Berkefeld N and W filters that retained small bacteria.

Partially purified concentrates were active after storage for 4 weeks at 0° C.

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RED LEAF, AN APHID-BORNE VIRUS DISEASE OF FILAREE

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Three species of filaree, *Erodium moschatum* L'Her. (white-stem filaree), *E. cicutarium* L'Her. (red-stem filaree), and *E. botrys* Bertol. (broad-leaf filaree), which are among the most valuable and abundant of range forage plants in California[2] and are also important sources of pollen and nectar,[3] commonly incur a disease of the virus-yellows type which has been called red leaf. All are introduced species[2] as is also the other known host species, *Geranium dissectum* L. This paper presents the results of intermittent work stimulated by the unproved possibility that the red-leaf virus might be the cause of a disease in an economic crop.

All experiments were conducted under controlled conditions in glasshouses. Standard techniques and equipment were used in handling plant materials and insects. *Erodium moschatum* was used as a test host exclusively, except in some of the later transfers included in the virus retention test, when it became necessary to use a small number each of *E. cicutarium*, *E. botrys*, and *Geranium dissectum* plants because of unexpected depletion of *E. moschatum* stock.

SYMPTOMS

Diseased plants may be recognized in the field by the reddish older leaves, the dwarfed, mildly chlorotic, erect, younger leaves with slightly crinkled, outward-cupped leaflets, and the stunted condition of the plants. In appearance the red-leaf disease somewhat resembles the disease caused by the California aster-yellows virus but may be distinguished by the absence of the secondary branching, virescence, phyllody, inward-cupped leaves, and marked chlorosis that are caused by the aster-yellows virus in filaree.

Symptoms of the red-leaf disease on experimentally infected plants are typical of field-infected plants. On *Erodium moschatum* symptoms are first apparent on immature leaves and consist, in nearly simultaneous appearance, of mild veinclearing; outward curvature of the petiole; inward cupping of leaflets (Fig. 1, d), which tend to be slightly linear and folded inward along the midrib so that the opposite leaf halves often are appressed (Fig. 1, e); mild chlorosis at leaflet margins extending inward between main veins (Fig. 1, f); and crinkling of lamina with the interveinal areas elevated above the troughlike main and lateral veins (Fig. 1, g). Curving, zigzagging, or twisting of petioles may follow closely. After the onset of symptoms new growth is stunted and dwarfed (Fig. 1, b). As the leaf

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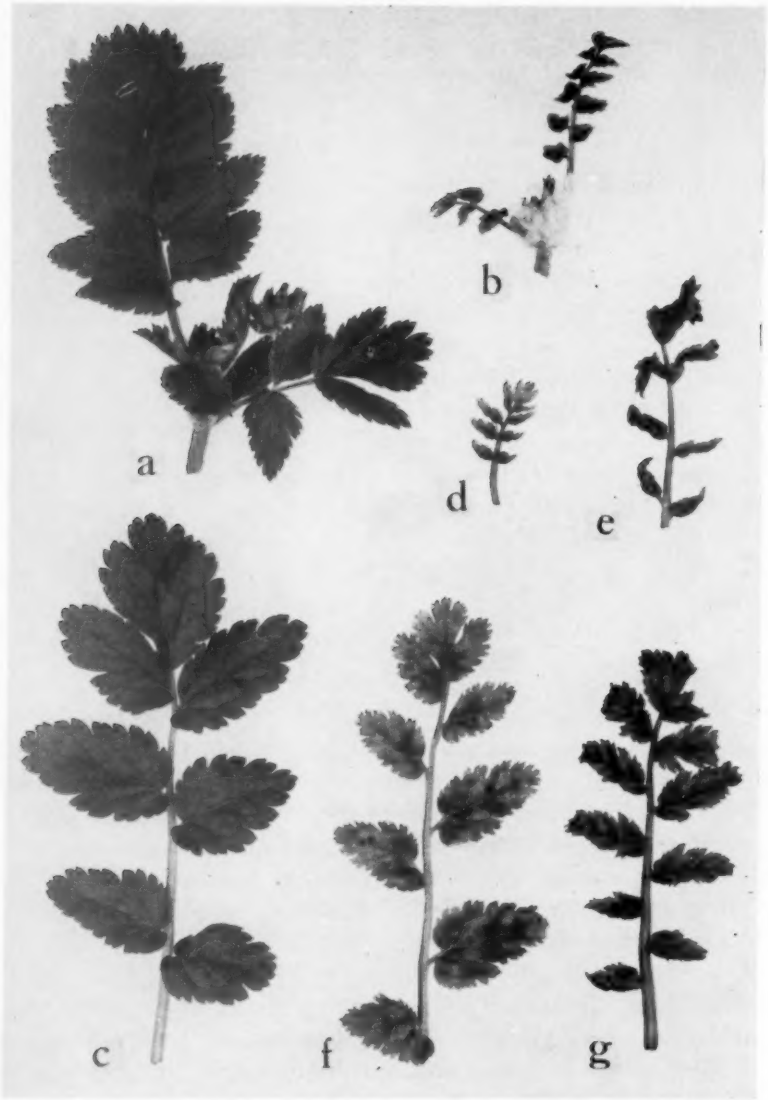

FIG. 1. *Erodium moschatum*, experimentally infected with filaree red-leaf virus disease. Tip of shoot from healthy plant (a) and one from diseased plant (b), showing dwarfing. Healthy leaf (c), and diseased leaves (d, e, f, and g), showing types of chlorosis, upward cupping, and malformation of leaflets.

matures the inward cupping of the leaflets changes to an outward cupping, the petioles tend to straighten and assume a stiff upright position, leaf texture becomes brittle, and the previously chlorotic marginal and interveinal areas gradually assume a characteristic reddish coloration from which the name of the disease is derived.

As the disease progresses the usual effect on the reproductive parts is one of increasing severity ranging from sterility to complete suppression of

floral development. Flowers may be produced on diseased plants but all organs usually are dwarfed in some degree, especially the stamens which may have chlorosis and short filaments, and be apparently devoid of pollen. Stunting and dwarfing usually become severe, and frequently a rosetted appearance develops after a time.

On young vigorous plants symptoms usually first appear in 7 to 10 days after infection although a range in the incubation period of from 5 to 26 days was observed.

TRANSMISSION TESTS

Mechanical inoculation. Efforts to transmit the red-leaf virus from naturally and experimentally infected *Erodium moschatum* to healthy *E. moschatum* by the carborundum method of juice inoculation failed. Twenty test plants inoculated in lots of five plants with expressed juice from each of four diseased plants remained healthy.

Tests with aphids.[4] The first test made to determine whether the red-leaf virus might be insect-borne was successful. The aphids present naturally on several diseased *Erodium moschatum* plants found in the field were transferred to healthy seedlings, several of which subsequently developed symptoms typical of the disease on the field plants.

Some of the more common species of aphids that were found to occur naturally on *E. moschatum* at Berkeley were tested for their ability to transmit the virus and the results are summarized in table 1. *Capitophorus fragaefolii* (Cockerell) is the only one of these species of aphids that was not found on filaree.

TABLE 1.—*Results of tests with six species of aphids for their ability to transmit the filaree red-leaf virus from diseased to healthy Erodium moschatum*

Species of aphid	Number of aphids per plant	Number of plants inoculated	Number of plants infected
Macrosiphum geranicola	1	38	19
Do	10 to 20	26	26
M. solanifolii	15 to 20	13	11
Myzus solani	1	9	0
Do	10 to 20	18	9
M. ornatus	15 to 20	10	0
M. persicae	10 to 25	27	0
Capitophorus fragaefolii	10	5	0

Macrosiphum geranicola (Ris Lambers) was one of the most common species found on white-stem filaree. It appeared to be an efficient vector of the virus, well adapted to filaree, and readily handled under caged conditions. This aphid was therefore used almost exclusively in carrying out the host-range tests. *M. solanifolii* (Ashmead) and *Myzus solani* (Kaltenbach) transmitted the virus, but neither *Myzus ornatus* (Laing), *M. persi-*

[4] The author is indebted to Dr. C. W. Anderson for the determination of *Macrosiphum geranicola*, and to Professor E. O. Essig who determined all other species of aphids during the course of this investigation.

cae (Sulzer), nor *Capitophorus fragaefolii* gave any indication in these tests that they were vectors.

EXPERIMENTAL HOST RANGE

A number of plants other than *E. moschatum* were experimentally inoculated with the red-leaf virus using from 10 to 25 infective aphid vectors per plant. *Macrosiphum geranicola* was the vector used for most of the tests but both *M. solanifolii* and *Myzus solani* were also used. Control inoculations to *E. moschatum* were made simultaneously to check on the infectivity of the vectors.

In the tests made with demonstrated infective vectors infection was obtained on *Erodium botrys* (five plants inoculated, five infected), *E. cicutarium* (5/5), and *Geranium dissectum* (5/5).

No infection was obtained on ten plants of *Apium graveolens* L. var. *dulce* DC., ten *Beta vulgaris* L., five *Brassica oleracea* L. var. *botrytis* L., three *Callistephus chinensis* Nees., six *Cicer arietinum* L., five *Cucumis sativus* L., four *Cucurbito Pepo* L., ten *Fragaria chiloensis* Duch. var. *ananassa* Bailey, five *Glycine max* Merr., six *Lathyrus odoratus* L., seven *Lycopersicon esculentum* Mill., five *Mathiola incana* R. Br., sixteen *Medicago sativa* L., two *Nicotiana glutinosa* L., two *N. tabacum* L., eight *Pelargonium hortorum* Bailey, five *Phaseolus vulgaris* L., ten *Pisum sativum* L., five *Plantago major* L., five *Spinacia oleracea* L., five *Trifolium pratense* L., six *Trifolium repens* L., five *Vicia faba* L., six *V. villosa* Roth, five *Vigna sinensis* Endl., or three *Vitis californica* Benth.

The symptoms produced on the three hosts were very similar, appeared in similar sequence, and were typical of those described for *E. moschatum*. Stunting and dwarfing were not so severely expressed on *E. cicutarium*, while affected leaves of *E. botrys* tended to assume a horizontal rather than the vertical position as in *E. moschatum* and *E. cicutarium*. The disease was very severe on *G. dissectum*. Growth was almost completely inhibited and the new organs formed were vestigial (Fig. 2, B, b). A most striking effect was expressed on the first leaves to show infection: the lamina became cupped and rolled outward giving the appearance of a ball (Fig. 2, B, f).

No symptoms that could be attributed to infection with the red-leaf virus were observed on any of the other 26 species tested. This would seem to indicate that the red-leaf virus is not one commonly met with among commercial crops and apparently causes disease primarily in geraniaceous hosts.

Recovery of the virus to *E. moschatum* was not attempted from any of the experimentally infected host plants. Evidence that *E. cicutarium*, *E. botrys*, and *Geranium dissectum* are hosts of the virus is therefore essentially circumstantial. However, every plant to develop symptoms did so within 6 to 19 days after inoculation, an incubation period comparable to that on *E. moschatum*. Symptoms were consistent within each species and very similar to the symptoms observed on *E. moschatum*, and, in *E. cicu-*

tarium and *E. botrys*, closely resembled symptoms observed on naturally diseased plants in the field. The red-leaf disease has not been observed on *G. dissectum* under natural conditions.

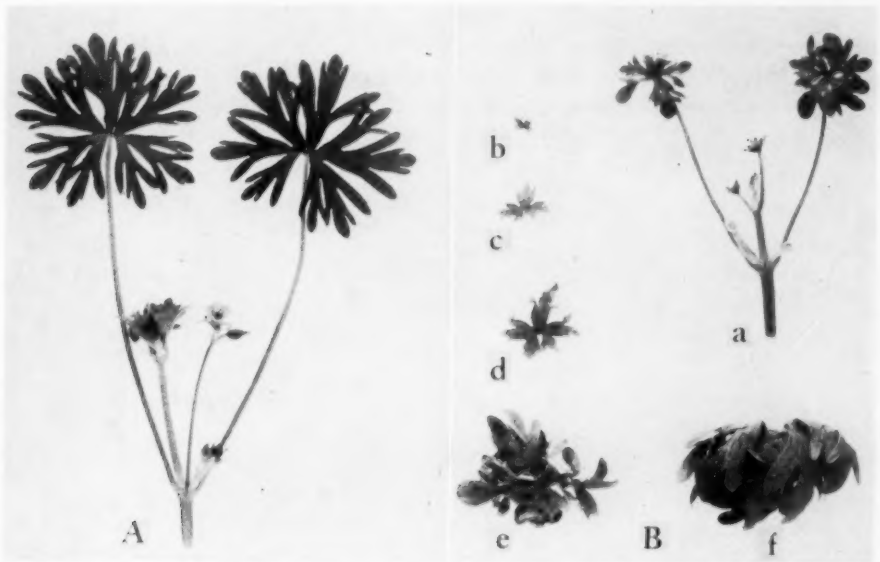

FIG. 2. *Geranium dissectum* experimentally infected with filaree red-leaf virus disease. A. Tip of shoot from healthy plant. B. Tip of stunted, diseased shoot (a) with dwarfing of leaves; diseased leaves (b, c, d, and e) in various advanced stages of infection with different degrees of dwarfing, chlorosis, and malformation; (f) diseased leaf in early stage of infection, ball-like appearance caused by outward cupping of the lamina.

VECTOR-VIRUS RELATIONSHIPS

No attempt was made to determine the minimum acquisition period or the minimum latent period. The aphid stock colonies used for most of the host-range tests were maintained on diseased filaree. In tests involving noninfective aphids the acquisition and test-feeding periods were each 24 hr. or longer. The shortest combined acquisition-test feeding period was 3 days and was above the minimum required as virus transmission resulted.

Retention of virus. Preliminary evidence indicated that the virus might be relatively persistent in its aphid vectors. A test was carried out to determine how long the vector *Macrosiphum geranicola* retained the ability to transmit the virus following removal from a virus source.

Two populations of previously noninfective *M. geranicola* were placed on separate red-leaf plants which had been infected experimentally by aphid vectors. After remaining on the diseased plants for a virus acquisition feeding of 5 days in the case of one population and 6 days in the other, each population was divided into five lots of 20 aphids each. These lots were transferred on March 5 to healthy test seedlings for 24 hr. and to successive healthy seedlings daily thereafter until all aphids were dead. Be-

cause of an unexpected shortage of available test plants some of the lots were combined on the 8th, 9th, 13th, and 23rd days.

The results of the test (Table 2) show that the virus was transmitted to

TABLE 2.—*Results of tests to determine the period of retention of the filaree red-leaf virus in the aphid vector, Macrosiphum geranicola. Infective aphids transferred at daily intervals to healthy filaree plants*

Day	Population A 6-day acquisition period					Population B 5-day acquisition period				
	Lot 1	Lot 2	Lot 3	Lot 4	Lot 5	Lot 6	Lot 7	Lot 8	Lot 9	Lot 10
1	20+[a b]	20+	20+	20+	20+	20+	20+	20+	20+	20+
2	7+	20+	17+	12+	2–[c]	20+	17+	19+	16+	14+
3	7+	17+	15+	11+	2–	18+	16+	17+	14+	13+
4	7+	16+	13+	9+	2+	18+	16+	17+	14+	10+
5	6+	16+	13+	9+	2+	18+	16+	16+	14+	10+
6	5+	15+	13+	9+	2+	11+	13+	13+	12+	7+
7	5+	14+	12+	9+	2+	7+	8+	10+	12+	7+
8	5+	13+	8+	8+	2+	**	11+	9+	15+	**
9	**[d]	15+	**	17+	**		8+	6+	9+	
10		15+		13+			5+	dead	5+	
11		15+		13+			2+		5+	
12		14+		11+			1+		3+	
13		14+		10+			**		5–	
14		13+		9+					1+	
15		11+		8+					1+	
16		8+		8+					dead	
17		7+		8+						
18		7+		7+						
19		7+		7+						
20		7–		7+						
21		6+		7+						
22		6–		4–						
23		**		9–						
24				4–						
25				2–						
26				dead						

[a] Numbers represent the number of aphids transferred to a healthy plant.
[b] The plus sign designates tests which resulted in infection of the test plant.
[c] The minus sign designates tests which did not result in infection.
[d] The asterisk indicates that lots were combined on the indicated day: lot 1 with 2; lot 2 with 4; lot 3 with 4; lot 5 with 4; lot 6 with 7; lot 7 with 9; and lot 10 with 9.

every consecutive test plant except four until the 21st day. There were no infections from the 22nd to the 25th day, when the last remaining aphids died. Additional data would be necessary to determine the significance of the sudden failure of the aphids to cause infection following the 21st day. It is not known why aphids in lots 1 to 5 survived until the 25th transfer while those in lots 6 to 10 failed to survive longer than the 15th transfer.

SUMMARY

The symptoms of a virus disease of the yellows type that is of common occurrence on *Erodium* spp. in California are described.

The virus was transmitted by three species of aphids, namely, *Macrosiphum geranicola, Macrosiphum solanifolii,* and *Myzus solani.* Three

species of aphids, *Myzus persicae*, *Myzus ornatus*, and *Capitophorus fragaefolii* failed to transmit the virus.

The virus was not transmitted by means of juice inoculation.

The following plants were experimentally infected by means of aphid vectors: *Erodium moschatum*, *E. cicutarium*, *E. botrys*, and *Geranium dissectum*. The disease was not recognized in 26 other plant species experimentally inoculated by means of infective aphid vectors.

The virus was retained in the vector *Macrosiphum geranicola* for 21 days following removal from virus source plants.

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SURFACE ROT OF PORTO RICAN SWEET POTATOES

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(Accepted for publication November 14, 1950)

A surface rot of Porto Rican sweet potatoes has become increasingly important in certain sections of Louisiana in recent years. It is found largely on sweet potatoes during and after storage. The lesions are somewhat circular and vary from minute size to 3 in. or more in diameter. On sweet potatoes that have been stored 3 to 5 months, the lesions usually are rather large. In some cases the decay is restricted to the portion outside the vascular ring, but frequently it is deeper. Occasionally the lesions resemble closely those of black rot caused by *Ceratostomella fimbriata* (Ell. & Hals.) Elliott (Fig. 1). In such cases, the disease can be definitely differentiated

FIG. 1. Surface rot on Porto Rican sweet potatoes that had been in commercial storage 5 months.

from black rot only by isolating the causal organism, which is a species of *Fusarium*.

Harter and Weimer[3] described a surface rot of sweet potatoes and at-

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[3] Harter, L. L., and J. L. Weimer. The surface rot of sweet potatoes. Phytopath. 9: 465–469. 1919.

tributed it to *Fusarium oxysporum* Schlecht. The disease on Porto Rican sweet potatoes in Louisiana differs considerably from their description and illustrations; the lesions are large, and ordinarily there are only one or two to a potato. The potatoes do not shrink excessively and do not become mummified unless secondary organisms, such as *Diplodia tubericola* (E. & E.) Taub. or *Sclerotium bataticola* Taub., are associated with the disease.

EXPERIMENTAL WORK AND RESULTS

For several years numerous isolations from surface-rot lesions generally have yielded cultures of *Fusarium*. Pathogenicity tests were made on Porto Rican sweet potatoes by puncturing the skin and inserting the mycelium and spores of different isolates obtained from surface-rot lesions. Inoculated sweet potatoes were kept in moist chambers in the laboratory. Eight to 10 days after inoculation, certain of the isolates had produced circular lesions (Fig. 2, B). When sweet potatoes with such lesions were stored commercially in ventilated, unheated houses, the lesions on certain of the potatoes enlarged and after 40 to 60 days in storage, typical surface-rot lesions had developed (Fig. 2, C and D).

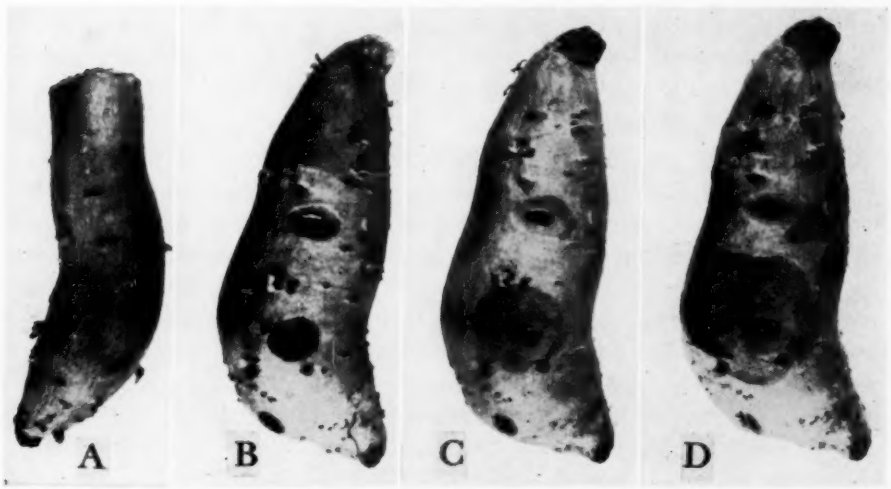

FIG. 2. Results of inoculation of sweet potatoes with *Fusarium* sp. isolated from surface-rot lesions. A, noninoculated check; B, C, and D, potatoes photographed 6, 24, and 44 days, respectively, after inoculation.

To determine the possible relationship of the sweet-potato stem-rot fungi (*Fusarium batatatis* Wr. and *F. hyperoxysporum* Wr.) to the fungus causing surface rot, isolates of the stem-rot fungus were puncture-inoculated into sweet potatoes as described above. No lesions were produced by the stem-rot fungus. In another test, sweet potatoes were grown in crocks of sterile soil inoculated in one series with a stem-rot isolate and in another series with a surface-rot isolate; the plants in the first series developed typical stem-rot symptoms, but those in the other series failed to develop stem-

rot symptoms. Dark lesions had appeared on some of the roots of the latter plants, but there was no evidence of vascular discoloration or other indications of stem rot.

In a further study of the pathogenicity of the Fusarium causing surface rot of Porto Rican sweet potatoes, 6-in. clay pots of sterile soil were inoculated with seven isolates of the organism and with an isolate of the sweet-potato stem-rot fungus. The soil in each pot was mixed with 100 gm. of inoculum consisting of a 10-day-old growth of the Fusarium isolate on sterilized oats. Four cuttings of Porto Rican sweet potatoes were planted in two pots of soil inoculated with each isolate, and four seed pieces of Triumph Irish potatoes were planted in two other pots. The soil was inoculated and planted on January 26, 1950. The pots were watered daily and kept in the greenhouse at temperatures ranging between 80° and 90° F. until they were dug and examined on April 3, 1950. There was no evidence of pathogenicity of the isolates on Irish potato. On sweet potato, the stem-rot isolate was very pathogenic, killing three of the plants and causing vascular discoloration in the fourth; no lesions were evident on the roots of the latter plant. One of the surface-rot isolates failed to produce lesions on the sweet-potato roots. Five of the isolates caused small lesions on the rootlets. The seventh isolate caused some die-back of rootlets and lesions on the larger roots. However, none of the surface-rot isolates caused sufficient injury to the sweet-potato plants to result in noticeable differences in outside appearance and vigor.

SUMMARY

A surface rot of Porto Rican sweet potatoes is described as being distinct from that reported on other varieties of sweet potatoes. Inoculation experiments have shown the causal agent to be a Fusarium. Isolates of the surface-rot Fusarium were relatively innocuous to young sweet-potato roots, although small root lesions were evident on plants grown in heavily inoculated soil. The surface-rot Fusarium did not cause stem rot of sweet potatoes, nor did the stem-rot Fusarium cause surface rot. Irish potatoes grown in soil inoculated with both the surface-rot and the stem-rot Fusaria did not become infected.

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INHERITANCE OF RESISTANCE TO LIMA-BEAN MOSAIC VIRUS IN THE LIMA BEAN

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(Accepted for publication November 15, 1950)

In 1945 in the vicinity of Beltsville, Maryland, Lima-bean mosaic (*Marmor cucumeris* var. *phaseoli* H.) was observed on Triumph (U. S. No. 343) Lima bean, but not on a sister line named Peerless (U. S. No. 243).[2] These two varieties are selections from a cross between Fordhook and Sieva Lima beans. Harter[3] reported that Lima-bean mosaic virus is infectious to the Sieva Lima, but not to the Fordhook Lima. The studies herein reported deal with the inheritance of resistance to this virus.

MATERIALS AND METHODS

The virus used in these studies was isolated from infected Lima beans growing at Beltsville, Maryland. It was maintained in a virulent condition by repeated transfers to the Triumph variety. Inoculum was taken from the new leaves of young infected plants approximately 10 days after inoculation.

All of the crosses and inoculations were made under controlled greenhouse conditions. Upon the discovery of the difference in reaction of Triumph and Peerless, some F_2 seed already on hand of the cross Triumph × Peerless and its reciprocal was studied. No F_1 seed of either cross was available at the time. Crosses between Fordhook and Sieva were made to obtain F_1 seed for testing.

The seed was planted in 4-in. pots and the plants were inoculated when the simple leaves were about three-fourths developed. Carborundum powder No. 320 was used as an abrasive. In susceptible plants vein necrosis developed in inoculated leaves within 5 days. This was followed by vein-clearing and downward cupping of young trifoliate leaves (Fig. 1, A). Petiole and stem necrosis developed on many of the plants, often resulting in death. Surviving plants were stunted and their leaves were mottled (Fig. 1, B).

After the first records were taken of the numbers of affected and symptomless plants, the latter were reinoculated. Frequently a third inoculation was made to insure infection of all plants not truly resistant. The surviving F_1 and F_2 plants were eventually transplanted into 10-in. pots for seed increase.

[1] Pathologist, Senior Pathologist, and Agricultural Aid, respectively, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture.

[2] Pryor, Dean E., and R. E. Wester. Relative resistance and susceptibility of U. S. 243 and U. S. 343 Lima beans to Lima-bean mosaic. Phytopath. 36: 170–172. 1946.

[3] Harter, L. L. Mosaic of Lima beans (*Phaseolus lunatus macrocarpus*). Jour. Agr. Res. [U. S.] 56: 895–906. 1938.

Seed from surviving F_2 plants, both susceptible and resistant, was planted and the resulting F_3 plants tested for reaction to the virus.

All data have been subjected to the χ^2 test for goodness of fit to certain theoretical ratios. This test was applied independently to F_2 families from individual F_1 plants, to F_3 families from F_2 plants, and to the totals of a number of tested families.

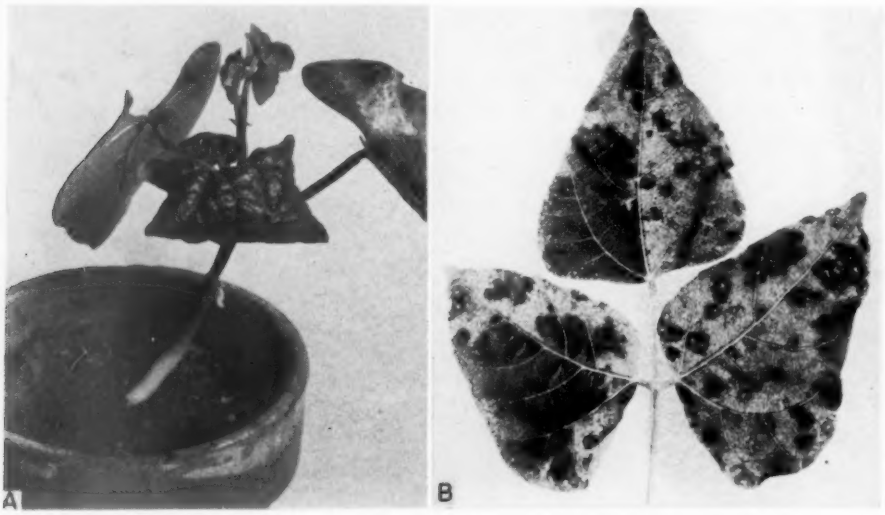

FIG. 1. Triumph Lima bean infected with Lima-bean mosaic. A. Veinclearing and downward cupping of young trifoliate leaves. B. Leaf from old, infected plant.

EXPERIMENTAL RESULTS

Reaction of parents. Fifteen plants of each parent variety were inoculated when the F_1, F_2, and F_3 populations were tested. All plants of Fordhook and Peerless varieties remained healthy, whereas all those of Sieva and Triumph became infected.

Reaction of F_1 generation. Eight plants from crosses between resistant and susceptible parents were tested. Four were from the cross Sieva × Fordhook and four from its reciprocal. All of the F_1 plants remained

TABLE 1.—*Reaction of F_2 plants when inoculated with Lima-bean mosaic virus*

Parentage of cross	Plants inoculated	Plants		χ^2 for 9 : 7 ratio[a]
		Resistant	Susceptible	
	Number	*Number*	*Number*	
Triumph × Peerless	66	39	27	0.217
Peerless × Triumph	99	54	45	0.117
Fordhook × Sieva	265	148	117	0.017
Sieva × Fordhook	105	58	47	0.044
Total	535	299	236	0.015

[a] 5 per cent point for 1 degree of freedom, 3.841.

healthy when inoculated with the virus. In subsequent tests, progenies grown from seed of each of these F_1 plants segregated for susceptibility and resistance, which indicated that none of the plants were selfs.

Reaction of F_2 generation. An analysis of the F_2 data (Table 1) shows that the resistance to the Lima-bean mosaic virus is dominant to susceptibility with an approximate 9 : 7 ratio, which indicates that two complementary factors control resistance to virus infection. The reciprocals behave in the same way. The χ^2 values indicate that the data fit the calculated 9 : 7 ratio closely for each of the four crosses. Out of a total of 535 plants, 299 were resistant and 236 susceptible. This segregation is a very close fit to a 9 : 7 ratio, with a χ^2 value of 0.015, far below the 5 per cent value of 3.841.

Reaction of F_3 generation. Of 63 F_3 families derived from resistant F_2 plants, 11 were homozygous resistant, 28 segregated into 3 resistant to 1 susceptible, and 24 segregated into 9 resistant to 7 susceptible plants (Table 2). This approximated a 1 : 4 : 4 ratio, with a χ^2 value of 2.856,[4] and

TABLE 2.—*Reaction of F_3 families descended from previously tested F_2 plants, when inoculated with Lima-bean mosaic virus*

Classification in F_3 generation	Families tested[a]	Plants tested	Plants	
			Resistant	Susceptible
	Number	*Number*	*Number*	*Number*
Homozygous resistant	11	417	403	14
Heterozygous 3 resistant; 1 susceptible	28	1083	854	229[b]
Heterozygous 9 Resistant; 7 susceptible	24	1222	716	506[c]
Homozygous susceptible	23	562	62	500
Total	86	3284		

[a] χ^2 for 1 : 4 : 4 distribution of healthy F_2 families, 2.856; 5 per cent point, 5.991.
[b] χ^2 for total population was 8.584; 5 per cent point, 3.841. The accumulated χ^2 was 23.199; 5 per cent point, 40.113 for 27 degrees of freedom.
[c] χ^2 for total population was 2.724; 5 per cent point, 3.841. Accumulated χ^2 was 29.485; 5 per cent point, 35.172 for 23 degrees of freedom.

failed to deny the two-factor hypothesis suggested by the behavior of the F_2 generation.

DISCUSSION

The results indicate that resistance to infection with the Lima-bean mosaic virus is controlled in the Fordhook and Triumph varieties by two complementary factors. The deficiency of susceptible plants in the 28 F_3 heterozygous families which segregated 3 : 1 could well be due to the small populations of many of the tested families. It was not possible to use large populations and in some cases progenies consisted of only 20 plants. A few susceptible plants escaping infection could have resulted in the classification of a family in the 3 : 1 instead of the 9 : 7 segregating class.

Considerable effort was made to prevent entwining of plants in the

[4] Five per cent point, 5.991.

greenhouse; however, it is probable that some slight mixture may have occurred during harvest. In five of the F_3 families, ranging in size from 33 to 50 plants and classified as homozygous resistant, from one to four susceptible plants were found. These families were not classified as heterozygous as the number of susceptible plants was not even close to a 9:7 or 3:1 fit. The 62 plants recorded as resistant in the 23 F_3 homozygous susceptible families probably merely escaped infection.

SUMMARY

In crosses between certain Lima-bean varieties resistant and susceptible to the Lima-bean mosaic virus, the F_1 plants from a cross between Fordhook (resistant) and Sieva (susceptible) varieties were resistant. A segregation of 9 resistant to 7 susceptible plants was obtained in the F_2 generation when the resistant variety was Fordhook or Peerless and the susceptible was Triumph or Sieva. The results for the F_3 generation substantiated those obtained in the F_2 generation.

The inheritance of mosaic resistance in Fordhook and Peerless varieties appears to be controlled by two dominant complementary factors.

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THE EFFECTS OF NITROGEN DEFICIENCY ON THE PROPERTIES OF TOBACCO-MOSAIC VIRUS[1]

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(Accepted for publication November 18, 1950)

In bacterial cultures the composition of the culture medium exerts a marked influence on the size of the bacteria (1, p. 81). So far as can be determined, no information is available regarding the influence of the composition of the host plant on size of virus particles.

It has previously been shown by Spencer (8) that ultracentrifugally purified tobacco-mosaic virus obtained from plants suffering from nitrogen deficiency shows reduced infectivity per weight of virus as compared with virus from plants grown in complete nutrient solution. *Phaseolus vulgaris* var. Early Golden Cluster was the assay plant employed by Spencer.

Since much evidence indicates that most of the infectivity is due to those virus particles around 280–300 mμ in length (4, 7, 9), work was carried out to determine whether nitrogen nutrition influences the size of virus particles and whether the reduced infectivity of virus from nitrogen-deficient plants is related to size of the particles.

Seeds of *Nicotiana tabacum* var. Turkish were germinated in sand, and the seedlings transplanted when in the 3- or 4-leaf stage to complete nutrient solutions in glazed earthenware crocks.

In one group of experiments (I), plants were inoculated when 6 in. high with tobacco-mosaic virus by rubbing three lower leaves of each plant with infectious juice. Ten days later, one-half of these plants were transferred to solutions deficient in nitrogen. The composition of the complete and nitrogen-deficient solutions is in Table 1.

TABLE 1.—*Composition of nutrient solutions*

Stock solution	Ml. stock/liter of nutrient solution	
	Complete	Deficient
M/1 KNO_3	7.0	0.0
M/1 $Ca(NO_3)_2$	3.5	0.0
M/1 KPO_3	1.0	1.0
M/1 $MgSO_4$	1.0	1.0
M/1 KCl	0.0	7.0
M/1 $CaCl_2$	0.0	3.5
A-5[a]	1.0	1.0
3 per cent $FeNH_4$ citrate	0.2	0.2

[a] Micronutrient solution as described in literature reference 2.

In another group of experiments (II), when the seedlings were 10 in. high, one-half were transferred to nitrogen-deficient solution. Ten days

[1] Part of this study was undertaken while the author held a pre-doctoral fellowship from the U. S. Public Health Service at the University of California, Berkeley.

later, all plants were inoculated with virus, and immediately topped by removing all growth above the uppermost inoculated leaf. All cultures were given supplementary aeration.

Inoculated leaves were harvested 20 and 14 days after inoculation in Groups I and II, respectively. Virus was extracted and purified, using essentially the methods employed by Spencer. These involved at least two alternate low- and high-speed centrifugations, and the use of pH 7 phosphate buffer as a suspending medium to minimize end-to-end aggregation of virus.

An RCA type B electron microscope, at a magnification of approximately 8,000 diameters, was used to obtain micrographs of purified virus. These were subsequently enlarged 5 times, and the sizes of the virus particles determined after their magnified images were measured with calipers. The results of these measurements for Groups I and II are combined in table 2,

TABLE 2.—*Effect of nitrogen status of host on virus size*

Nitrogen status	No. of particles measured	Percentage of particles		
		< 225 mμ long	225–356 mμ long	> 357 mμ long
+	1521	25.8	67.0	7.2
−	1310	15.5	72.6	11.9

since there were no significant differences between the two.

It is believed that the difference in the percentage of particles between 225 and 357 mμ long, presumably the range in which most of the infective particles are found, can in no way account for the activity differences reported by Spencer. The latter found that, on the average, virus from normal plants was twice as infective as virus from nitrogen-deficient plants. Apparently size relations are not involved. Nitrogen deficiency did not produce a significant change in the size of the virus particles.

The infectivity of purified virus was compared on both *Phaseolus vulgaris* var. Early Golden Cluster and *Nicotiana glutinosa*. The half-leaf local lesion method of Youden and Beale (10) was employed for assay with *N. glutinosa*, and the modified local lesion procedure recommended by Spencer was followed for bean. Results of the activity assays are summarized in table 3.

The results with bean are essentially in agreement with those obtained

TABLE 3.—*Virus activity on two assay hosts*

Host	Activity of N-deficient virus[a]			
	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Nicotiana glutinosa	84.8	47.2	78.0	100.0
Phaseolus vulgaris	32.5	24.0	.....	42.5

[a] Activity is expressed as a percentage of the activity of the normal virus.

by Spencer, i.e., a reduced infectivity of "deficient" virus as compared with "complete" virus. However, no consistent reduction in infectivity of "deficient" virus toward *N. glutinosa* was found.

Similar host-dependent differential infectivity has been reported for certain strains of tobacco mosaic virus (3, 5), and for certain chemical derivatives of this virus (6).

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THE PATHOGENICITY OF RACES 1 AND 2 OF FUSARIUM OXYSPORUM F. LYCOPERSICI

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The search for tomatoes resistant to Fusarium wilt began as early as 1905. For many years investigators confined themselves to individual plant selection within varieties of *Lycopersicon esculentum* Mill., and progenies of intervarietal crosses. In 1939 Bohn and Tucker[2] described the resistance of *L. pimpinellifolium* Mill. (Accession 160[3]) to *Fusarium oxysporum* f. *lycopersici* (Sacc.) Snyder & Hansen. They studied the inheritance of resistance in hybrids of *L. esculentum* × *L. pimpinellifolium* and found that resistance to the fungus was controlled by a single dominant gene. In Bohn and Tucker's tests *L. pimpinellifolium* proved to be highly resistant to 39 isolates of the tomato wilt fungus obtained from various parts of the world. Wellman and Blaisdell[4] found it resistant to 29 isolates. In 1945 Alexander and Tucker[5] reported that plants possessing the *L. pimpinellifolium* factor for resistance were susceptible to an isolate of the fungus obtained in Ohio. They considered this isolate, Ohio-39, a new physiologic race.

In the present study, isolates nonpathogenic to plants possessing the *L. pimpinellifolium* factor for resistance, and pathogenic to plants which lack this factor, are referred to as race 1. Those pathogenic to plants with the *L. pimpinellifolium* resistance factor, as well as to plants without the factor, are referred to as race 2. This study was undertaken to determine the prevalence, pathogenicity, and possible origin of this new race.

MATERIALS AND METHODS

The technique for the pathogenicity tests conducted in 1944 and 1945 was as follows: Inoculum was prepared by growing the isolates on sterile oats in 1-pt. jars. When the cultures were from 2 to 5 weeks old a suspension of spores and mycelial fragments was prepared by adding 1 qt. tap water to each pint of inoculum. After thorough mixing, the suspension was strained to remove the oats. Approximately 30 seeds were planted in steamed soil

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The writers wish to express their gratitude to Dr. C. M. Tucker for his advice throughout this investigation and in the preparation of the manuscript.

[2] Bohn, G. W., and C. M. Tucker. Immunity to Fusarium wilt in the tomato. Science 89: 603–604. 1939.

[3] Acc. 160 is referred to hereafter as *L. pimpinellifolium*.

[4] Wellman, F. L., and Dorothy J. Blaisdell. Difference in growth characters and pathogenicity of Fusarium wilt isolations tested on three tomato varieties. U. S. Dept. Agr. Tech. Bul. 705. 1940.

[5] Alexander, L. J., and C. M. Tucker. Physiologic specialization in the tomato wilt fungus *Fusarium oxysporum* f. *lycopersici*. Jour. Agr. Res. [U.S.] 70: 303–313. 1945.

in 8-in. clay pots and inoculated with 200 ml. of the fungus suspension, poured over the seeds before they were covered with soil.

In the 1946 experiments, using inoculum prepared in the manner described above, roots of 2- to 3-week-old seedlings were dipped in the fungus suspension. The seedlings were grown in flats of steamed soil and transplanted after inoculation to 8-in. clay pots of sterile soil, 20 to a pot. The experiments were duplicated; replications were placed on separate benches in the same greenhouse. The air temperature was maintained as near 80° F. as possible; fluctuations were never great.

Plants were examined at 3-day intervals, and all having external symptoms of wilt were removed and recorded. In the first two experiments, final records were taken 50 to 70 days after seeds were planted. In plants inoculated by dipping roots in a fungus suspension the symptoms developed more rapidly, and final records were taken 26 to 34 days after transplanting.

Plants with no external symptoms of wilt or vascular discoloration in the stem at the ground line were classed as "healthy"; but when vascular browning occurred, the term "vascular discoloration" was applied. Plants were classed as "wilted" when they died from disease or had obvious external symptoms of disease. Isolates were tested on three tomato varieties: Bonny Best, very susceptible to both races 1 and 2; S-39, a third backcross hybrid of *Lycopersicon esculentum* × *L. pimpinellifolium* (selfed for eight or more generations, homozygous for resistance to race 1 and very susceptible to race 2); and *L. pimpinellifolium*, highly resistant to race 1 and moderately susceptible to race 2.

PATHOGENICITY TESTS OF ISOLATES

During September 1944, 55 isolates of the tomato-wilt organism were isolated from diseased tomatoes growing in a field on the University farm near Midway, Missouri. This field was infested that year and the preceding year with three virulent single-spore isolates of race 1: F-7, I-6, and I-10. Twenty-nine isolates were obtained from the following susceptible or wilt-tolerant commercial varieties: Indiana Baltimore, Marglobe, Rutgers, Garden State, Stokesdale, and Early Stone. Isolates were obtained from 26 hybrids of *Lycopersicon esculentum* × *L. pimpinellifolium:* eight were homozygous for resistance to race 1, two were heterozygous, eight were from progenies segregating for resistance and susceptibility, and eight were from homozygous susceptible progenies. The pathogenicity of these isolates was compared with that of F-7, I-6, and I-10. A summary of the results obtained is in table 1.

The 29 isolates obtained from commercial varieties produced from 23 to 100 per cent infection in Bonny Best, 0 to 6.1 per cent infection in S-39, and 0 to 1.5 per cent in *L. pimpinellifolium*. All were classified as race 1. All isolates obtained from plants homozygous for resistance to race 1 were capable of infecting both S-39 and *L. pimpinellifolium*, producing from 22

to 76.3 per cent infection in S-39 and 8.3 to 37.5 per cent infection in *L. pimpinellifolium*, and were classified as race 2. Of the isolates obtained from segregating progenies, six proved to be race 1 and two were race 2. The two isolates obtained from plants heterozygous for resistance to race 1 proved to be race 2. The gene for resistance to race 1 is dominant, therefore heterozygous plants are as highly resistant as are homozygous plants. Race 2 isolates were obtained from all plants known to be highly resistant to race 1, and race 1 isolates were obtained from all plants known to be susceptible to both races.

TABLE 1.—*Percentage infection of three tomato varieties by isolates of Fusarium oxysporum f. lycopersici*

Source of isolate	No. of isolates	Race	Test plants	Total plants	Percentage vascular discoloration	Percentage wilted
Commercial varieties	29	1	B.B.[a]	888	7.6	61.7
			S-39	1363	0.7	0.6
			L.P.	1749	0.1	0.0
Hybrids, homozygous susceptible to race 1	8	1	B.B.	232	7.3	56.5
			S-39	384	0.5	0.3
			L.P.	481	0.2	0.2
Segregating hybrids	6	1	B.B.	201	8.5	55.7
			S-39	287	1.1	0.3
			L.P.	358	0.0	0.0
Segregating hybrids	2	2	B.B.	65	6.2	32.3
			S-39	90	11.1	18.9
			L.P.	120	14.2	8.3
Heterozygous hybrids	2	2	B.B.	66	21.2	51.5
			S-39	84	26.2	44.0
			L.P.	116	16.4	8.6
Hybrids, homozygous resistant to race 1	8	2	B.B.	259	8.1	49.4
			S-39	357	22.4	26.3
			L.P.	474	12.2	3.8
Stock cultures F-7, I-6, I-10	3	1	B.B.	99	7.1	45.5
			S-39	147	0.0	0.0
			L.P.	186	0.0	0.0

[a] B.B., Bonny Best; S-39, hybrid of *L. esculentum* × *L. pimpinellifolium*, homozygous resistant to race 1; L.P., *L. pimpinellifolium*.

The tomato strain S-39 was more tolerant of race 2 isolates than Bonny Best. Although the percentage of infected plants was nearly the same in both, the number of Bonny Best plants having external symptoms was significantly higher. Race 2 was not more pathogenic to Bonny Best than race 1. The difference in pathogenicity, therefore, appears to be qualitative rather than quantitative.

Several isolates of race 1 caused infection in a few plants of S-39 and *L. pimpinellifolium*, but later tests indicated that this was not due to greater virulence. Porte and Wellman[6] also noted that none of the lines of *L. pimpinellifolium* tested were completely immune.

[6] Porte, William S., and F. L. Wellman. Development of interspecific tomato hybrids of horticultural value and highly resistant to Fusarium wilt. U. S. Dept. Agr. Circ. 584. 1941.

In September, 1945, six isolates were obtained from diseased hybrid tomatoes growing in a field which had been infested annually for 5 years with the race 1 isolates F-7, I-6, and I-10. Hybrid tomatoes had been grown there each season during that period. Isolates F-7, I-10, Ohio 39 (the Ohio race 2 isolate) and the six new isolates were tested during the winter. The results are summarized in table 2.

TABLE 2.—*Percentage infection of three tomato varieties by isolates of Fusarium oxysporum f. lycopersici*

Source of isolate	Race	Tomato variety[a]	Total plants	Percentage vascular discoloration	Percentage wilted
Hybrid, homozygous susceptible to race 1[b]	1	B.B.	46	0.0	97.8
		S-39	50	0.0	0.0
		L.P.	43	0.0	0.0
Segregating hybrid	2	B.B.	46	2.2	95.6
		S-39	46	2.2	97.8
		L.P.	35	48.6	17.1
Heterozygous hybrid	2	B.B.	49	10.2	89.8
		S-39	49	18.4	81.6
		L.P.	49	18.4	12.2
Heterozygous hybrid	2	B.B.	35	0.0	100.0
		S-39	39	10.3	82.0
		L.P.	44	20.4	2.3
Heterozygous hybrid	2	B.B.	53	3.8	94.3
		S-39	45	26.7	64.4
		L.P.	43	14.0	0.0
Hybrid, homozygous resistant to race 1	2	B.B.	41	0.0	100.0
		S-39	44	6.8	93.2
		L.P.	44	31.8	18.2
Ohio 39	2	B.B.	46	2.2	97.8
		S-39	48	2.1	93.7
		L.P.	47	19.2	19.1
F-7	1	B.B.	40	0.0	100.0
		S-39	49	6.1	0.0
		L.P.	49	0.0	0.0
I-10	1	B.B.	45	8.9	91.1
		S-39	53	1.9	0.0
		L.P.	45	0.0	0.0

[a] B.B., Bonny Best; S-39, hybrid of *L. esculentum* × *L. pimpinellifolium*, homozygous resistant to race 1; L.P., *L. pimpinellifolium*.

[b] Each entry represents one isolate.

The one isolate obtained from a hybrid plant, homozygous susceptible to race 1, proved to be race 1. Race 2 was isolated from one plant of a segregating progeny, from three plants heterozygous for resistance to race 1, and from one plant homozygous for resistance to race 1. The Missouri race 2 isolates and the Ohio isolate had similar types of pathogenicity. The stock cultures F-7 and I-10 appeared to be more pathogenic to both Bonny Best and S-39 than in the previous tests. This may have been due to more favorable environmental conditions for infection.

During August 1946, 45 isolates were obtained from third- and fourth-generation hybrids which were segregating for resistance to race 1. These

plants were growing in plots not previously planted to tomatoes. At the time of setting, the plants were inoculated with cultures of F-7 and I-10. In addition to the 45 isolates obtained in 1946, two of the more pathogenic race 2 isolates obtained in the two previous years (4-B-17 and 5-B-56), a reisolate of the Ohio isolate (designated hereafter as A-1-3), and three race 1 isolates (F-7, I-6, I-10) were tested. The 1944 and 1945 tests had been made with mass transfer cultures and the possibility existed that some of them were mixtures of both races. To eliminate this possibility, only subcultures of single-spore isolations were used in this test. The results are in table 3.

TABLE 3.—*Percentage infection of three tomato varieties by isolates of Fusarium oxysporum f. lycopersici*

Source of isolate	No. of isolates	Race	Test plants[a]	Total plants	Percentage vascular discoloration	Percentage wilted
Segregating progenies	44	1	B.B.	2117	3.9	91.5
			S-39	2091	2.2	1.9
			L.P.	2162	0.0	0.0
Segregating progeny	1	2	B.B.	48	0.0	100.0
			S-39	50	16.0	72.0
			L.P.	50	34.0	2.0
Stock race 2 cultures	4	2	B.B.	192	8.3	91.2
			S-39	186	24.2	59.1
			L.P.	198	29.8	9.1
A-1-3 (Ohio 39 reisolate)	1	2	B.B.	50	6.0	94.0
			S-39	49	12.2	87.8
			L.P.	50	22.0	28.0
Stock race 1 F-7, I-10	2	1	B.B.	95	5.3	92.6
			S-39	97	4.1	4.1
			L.P.	100	0.0	0.0

[a] B.B., Bonny Best; S-39, hybrid of *L. esculentum* × *L. pimpinellifolium*, homozygous resistant to race 1; L.P., *L. pimpinellifolium*.

Of the 45 isolates obtained from segregating *Lycopersicon esculentum* × *L. pimpinellifolium* progenies, only one possessed the race 2 type of pathogenicity. The behavior pattern of race 1 cultures was about the same in this test as in the previous ones. The percentage of infection was slightly higher in S-39. This apparent increase in pathogenicity may have been due to the more rigorous method of inoculation. *L. pimpinellifolium* proved as resistant to race 1 cultures as in previous tests.

During the course of the preceding experiment, isolations were made from 20 S-39 plants which had been inoculated with race 1 cultures and which developed disease symptoms. These reisolates were tested, using the same method. The results are in table 4. These reisolates from S-39 plants were no more pathogenic to S-39 and *L. pimpinellifolium* than the original cultures. This demonstrates that race 1 will occasionally infect plants possessing the *L. pimpinellifolium* resistance factor, when heavily inoculated

and maintained under near-optimum conditions, but that this does not necessarily indicate a change in pathogenicity.

TABLE 4.—*Infection of three varieties of tomatoes by reisolates of Fusarium oxysporum f. lycopersici, race 1, from infected S-39 plants*

Inoculum	Number of reisolates	Test plants[a]	Total plants	Percentage vascular discoloration	Percentage wilted
Eighteen isolates from segregating progenies	18	B.B.	900	4.7	90.9
		S-39	900	0.3	0.0
		L.P.	900	0.1	0.0
F-7	2	B.B.	100	6.0	89.0
		S-39	100	2.0	0.0
		L.P.	100	0.0	0.0
F-7 (Stock culture, check)		B.B.	100	2.0	94.0
		S-39	100	1.0	0.0
		L.P.	100	0.0	0.0

[a] B.B., Bonny Best; S-39, hybrid of *L. esculentum* × *L. pimpinellifolium*, homozygous resistant to race 1; L.P., *L. pimpinellifolium*.

DISCUSSION

It is possible that the isolates of race 2 of *Fusarium oxysporum* f. *lycopersici* isolated from tomatoes growing on the University farm near Midway, Missouri, were introduced from some outside source. However, tomatoes had not been grown in these fields previously and they had both been infested annually, for 5 years in the case of one field and for 2 years in the other, with isolates of race 1. Therefore it seems probable that the race 2 isolates appeared as mutants or variants of the race 1 isolates with which the fields were infested. In a species so variable in type of growth and physiology, which in culture frequently undergoes saltation, the occurrence of mutants or variants capable of infecting varieties resistant to the parent strain would not be surprising. When resistant plants are grown where such variants are occurring, a host in which they can grow without competition is provided for them. If the resistant variety had not been available, the variants might not have survived, since they are probably no better adapted than the parent strain for parasitic growth in a host susceptible to all races of the fungus, or for saprophytic existence in the soil.

Tests of single-spore isolates indicated clearly the qualitative difference in pathogenicity between the two races. Although the race 1 isolates occasionally infected resistant strains of tomatoes, the percentage of infection was always much lower than that obtained with race 2 isolates. An isolate of intermediate pathogenicity which might represent a transition type has never been found. Furthermore, the tests with reisolates from resistant plants infected by race 1 indicate that the difference in pathogenicity is not due to an adaptive change induced by association with resistant plants. There is need for a thorough comparative study of the morphology and physiology of the two races.

SUMMARY

Substantiating evidence was obtained for the existence of physiological specialization in *Fusarium oxysporum* f. *lycopersici*. Two races are described and designated as races 1 and 2. Race 1 is described as nonpathogenic to plants possessing the *Lycopersicon pimpinellifolium* resistance factor. Isolates which are pathogenic to plants with the *L. pimpinellifolium* resistance factor, as well as to those without the factor, are designated as race 2.

The qualitative nature of the difference in pathogenicity between the races, the absence of intermediate types, and the fact that race 2 isolates were obtained from resistant hybrids grown in field plots known to be infested with race 1, indicate that race 2 represents a one-step transition from race 1.

Pathogenicity tests with reisolates of race 1 cultures from the resistant hybrid, S-39, indicate that changes in pathogenicity are not brought about by adaptation.

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PHYSIOLOGY AND PARASITISM OF SCLEROTIUM ROLFSII[1]

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Southern blight, caused by the fungus *Sclerotium rolfsii* Sacc., often causes severe losses on a wide variety of crops throughout the tropic zone and warmer parts of the temperate zones of the world. The disease was first reported and described by Rolfs (22) in 1892 on tomatoes in Florida. Later in the same year the pathogen was isolated and its pathogenicity proved (23). Since then *S. rolfsii* has been reported from practically all the southern States (2, 5, 6, 12, 28); and from Asia, Africa, Australia, and many islands, including Japan, Ceylon, Cuba, Hawaii, and the Philippines (28). It is probable that its occurrence is practically universal in those parts of the world where the winters are not sufficiently cold to kill out the mycelium and sclerotia in the soil.

Host-range studies were first undertaken by Rolfs (23, 24), who was aware of the widespread occurrence of the disease on a large number of species of plants. The host range has since been lengthened by contributions from numerous investigators (5, 6, 21, 27, 28).

Higgins (8) made the greatest contribution to the knowledge of the physiology and parasitism of *S. rolfsii*. He made a thorough study of the growth requirements of the pathogen, including nutritional requirements as well as the effects of temperature, light, and pH of medium. He also studied the physiological relationship between the pathogen and host in an attempt to explain the wide host range.

Higgins reported that the optimum temperature for growth of *S. rolfsii* on artificial media is 30°–35° C. and that little growth occurs below 20°. This high temperature requirement has repeatedly been confirmed by various authors with respect to growth on both artificial and natural media (3, 4, 9, 10, 14, 15, 25). Rolfs (24), McClintock (13), and Endo (3) observed that growth and activity of the pathogen in the soil is greatest in warm soils between 25° and 36° C. and that activity is negligible below 15° C. Endo (4), working with two other species of the genus *Sclerotium*, observed that temperature affected seedling infection of rice directly as it affected the growth of the pathogen.

MATERIALS AND METHODS

Plants were grown in ½-gal. glazed pots for the soil temperature studies and in 4-in. clay pots for all other studies. Plants were thinned to four or five per pot, spaced at least an inch apart so that the pathogen would not grow through the soil from one plant to another during the course of the test. Inoculum was prepared by growing the fungus on autoclaved, moist wheat seeds for 5 to 6 days. Infested seeds were air-dried and, if held in

[1] Studies conducted at Camp Detrick, Maryland, from April 1944 to September 1945.

cool dry storage, could be stored for many months with no apparent loss in viability of the fungus. A single dried infested wheat seed was placed adjacent to the stem of each plant and just beneath the soil surface.

Soil temperatures were controlled by placing the pots in constant-temperature water baths, with controls sufficiently sensitive so that normal variations did not exceed 2° C. above or below the desired temperature. In order to study the destruction of germinating seeds at several temperatures, glazed pots were partially filled with soil and were placed in the baths. As soon as the soil temperature reached the temperature of the bath, the seeds were placed on the surface of the soil and covered to a depth of about ½ in. with a mixture of sand and a culture of *S. rolfsii* on garden peat.

Four isolates of *S. rolfsii* were used in these studies: the B strain from sugar beet from California, the R strain from rice from Texas, the S strain from soybean from Georgia, and the G strain from garden beet from Georgia.

In all inoculation tests, data were recorded of the number of plants killed and the length of time elapsing between inoculation and the death of the plants. Plants were recorded as dead when the fungus had so destroyed the base of the plant that it could no longer support the plant in an upright position, or when obvious permanent wilting occurred. Observations were made at 24-hr. intervals. Usually a vigorous inoculum caused death of all inoculated plants, so that differences among treatments could be shown only by the differences in time elapsing between inoculation and death of the plants.

Vigor of the mycelium in the infested wheat seeds was determined by placing on a water agar plate 25 or more seeds selected at random from the dried sample of inoculum. The vigor was judged by the mean diameter of the colonies after 24 hours' incubation at 32° C. or by the percentage of seeds giving rise to colonies at least 15 mm. in diameter in the same period. The 15-mm. diameter was selected because numerous tests indicated that the mycelium in a seed capable of giving rise to a colony of this size in 24 hr. was also capable of killing young cowpea plants in greenhouse tests, whereas smaller colonies grew so slowly as to allow the plant to pass out of its most susceptible stage of growth and thus to survive the inoculation.

SURVIVAL OF MYCELIUM IN INFESTED WHEAT SEEDS

On September 1, 1944, a single lot of air-dried infested wheat seeds was mixed thoroughly, divided into 20 equal parts, and stored under conditions of controlled temperature and relative humidity. In an agar-plate test on September 1, 95 of 100 infested seeds tested contained mycelium capable of producing a colony 20 mm. in diameter in 24 hr. at 32° C., and five produced colonies between 15 and 20 mm. in diameter. The seeds were stored in sealed 1-qt. Mason fruit jars. Each jar contained a 50-ml. beaker of seeds and a beaker of sulfuric acid of that concentration required to maintain the relative humidity in the jar at the desired level. Four relative

humidities were maintained: 12, 25, 37, and 50 per cent, and five temperatures, 7°–8°, 20°, 27°, 32°, and room temperature, which varied between 20° and 30°. At monthly intervals, 25 seed samples were removed and plated on water agar plates to determine the number of seeds bearing viable mycelium and the vigor of the mycelium from the seeds. Data are in figure 1.

Mycelium in almost all seeds stored at 50 per cent relative humidity and 32° C. was dead at the end of 1 month. Seeds stored at 12 or 25 per cent

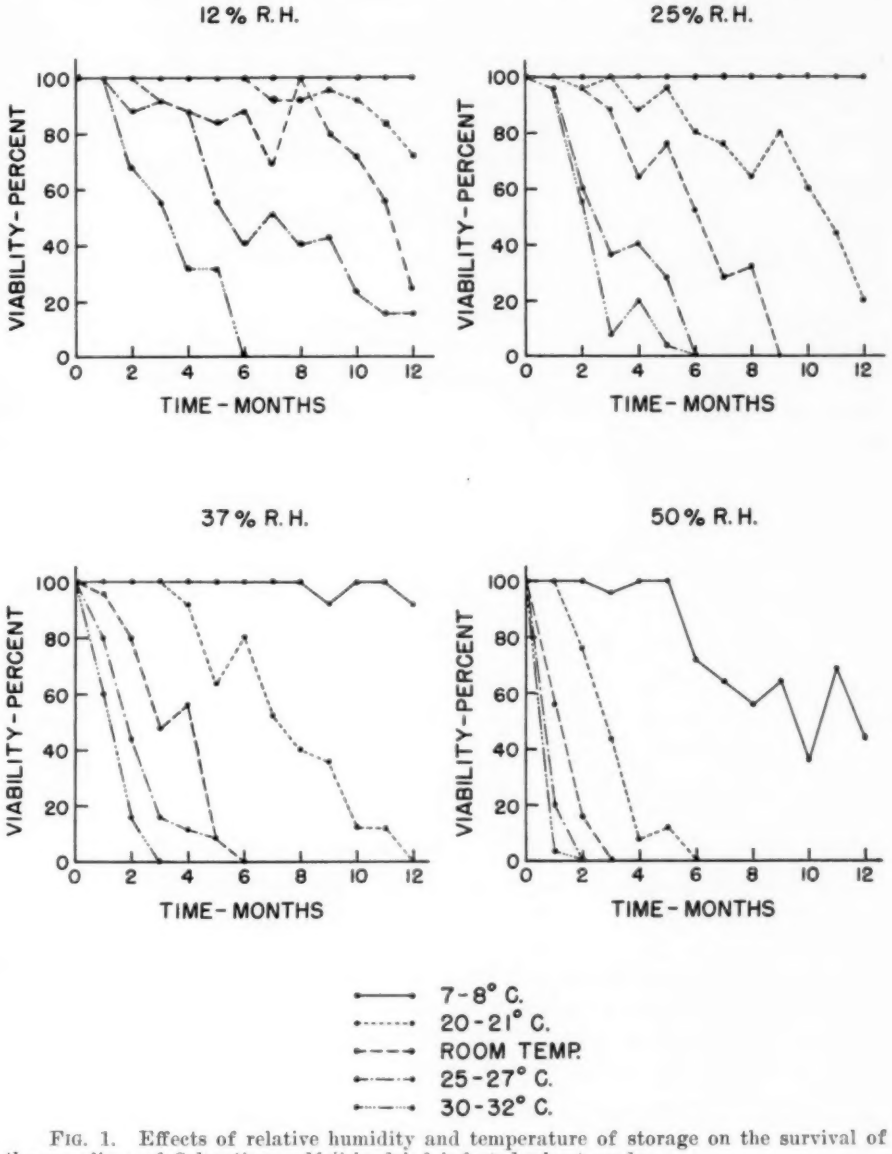

FIG. 1. Effects of relative humidity and temperature of storage on the survival of the mycelium of *Sclerotium rolfsii* in dried infested wheat seeds.

R. H. and 7°–8° produced vigorous colonies after a year of storage. Colonies produced from seeds stored at 25 per cent R. H. appeared slightly more vigorous than colonies from seeds stored at 12 per cent R. H. The difference was too small to indicate significance, however. Seeds stored at 7°–8° and 37 per cent R. H. produced a larger number of small weak colonies than those stored at 12 or 25 per cent R. H. and at the same temperature. The data in figure 1 are expressed as the percentage of the seeds containing viable mycelium, since any significant reduction in vigor of growth was followed quickly by a reduction in the number of seeds containing viable mycelium. Both temperature and relative humidity were important factors in determining the length of time the mycelium remained viable in the dried seeds.

FACTORS AFFECTING THE INFECTIVITY OF SCLEROTIUM ROLFSII

Soil temperature. The greatest number of young plants was killed in the shortest time at the higher soil temperatures used in these tests, namely 30°–35° C. Essentially all the inoculated plants were killed at these temperatures and the interval between inoculation and death was at a minimum. When the temperature was lowered to 25°, the only evident change was a slightly longer time elapsing between inoculation and the death of the plants. At 20° the time elapsing between inoculation and death was further increased and a few of the inoculated plants survived. As the temperature dropped below 20° the number of survivors and the time interval between inoculation and death both increased sharply (Table 1). The tem-

TABLE 1.—*Influence of soil temperature on the killing of young plants by Sclerotium rolfsii*

Temperature (°C.)	Number of plants dead on day following inoculation of plants[a]											Total plants killed	Days to death (average)
	1	2	3	4	5	6	7	8	9	10	11		
35	0	51	56	19	5	4	0	0	2	0	0	137	3.01
30	0	53	44	23	13	5	0	0	0	0	0	138	3.08
25	0	26	50	36	19	5	1	0	2	0	0	139	3.57
20	0	10	32	41	28	15	3	0	3	0	0	132	4.23
15	0	0	10	15	7	20	15	18	12	0	1	98	6.26

[a] Results from inoculation of 140 plants at each temperature—40 tomato, 40 soybean, 20 potato, 20 sugar beet, and 20 cowpea.

perature effects were about the same for all the five crops tested and all the data are grouped into a single table. The potato, which is better adapted to growth in cool soils, had the greatest increase in number of survivors as the temperature was reduced.

The effect of soil temperature on the prevention by *S. rolfsii* of emergence of seedlings from seeds planted in infested soil was very similar to its effect on young plants. Rice and soybean seeds were used in these tests. Rice and other grasses are highly resistant to this pathogen after the plant emerges from the soil, but the germinating seeds are susceptible to attack.

The maximum reduction in stand occurred at the higher temperatures, namely 25°–35° C. This was true both with rice and with soybean; although the degree of reduction with rice was smaller than that with soybeans. The stand improved as the temperature was reduced (Table 2).

TABLE 2.—*Emergence of rice and soybean plants from seeds planted in soil infested with Sclerotium rolfsii at several temperatures*

Soil temperature (°C.)	Stand from 100 seeds planted (average)		Percentage stand reduction
	Clean soil	Infested soil	
	Rice (one test)		
35	85.0	35.0	58.8
30	92.5	55.8	39.7
25	92.5	54.2	41.4
20	93.3	70.0	25.0
15	87.5	73.3	16.2
	Soybean (four tests)		
28	83.0	7.4	91.1
24	84.1	19.3	77.1
20	86.4	36.4	57.9
16	86.4	50.0	42.1
12	75.0	46.6	37.9

Soil moisture. Laboratory tests were made to determine the ability of the pathogen to grow and to attack germinating seeds, and the ability of seeds to germinate in relatively dry soils. A lot of moist river sand was mixed thoroughly and was dried slowly, with frequent stirring, in the laboratory. At intervals, as the sand dried, samples were removed. This procedure resulted in a series of samples of sand varying in moisture content from about 0.5 to about 3.0 per cent in the various replications of the test. Growth of rice and soybean seeds and of the fungus from infested seeds on this sand was observed at daily intervals.

No visible growth of the fungus or of the seeds occurred on sand containing 0.93 per cent or less moisture. Slight growth of the fungus occurred at 0.96 per cent, and good growth at 1.02 per cent moisture. At a moisture content of 1.34 per cent and higher the fungus grew vigorously over both the rice and the soybean seeds. Weak rice sprouts appeared in the control plate on sand containing 1.93 per cent, and vigorous rice and soybean sprouts appeared on sand with 2.05 per cent moisture. All seeds attacked by the pathogen failed to germinate.

The real values observed in this series of tests mean little and would vary widely with different soil samples. They do indicate, however, that *S. rolfsii* is capable of growing out from the infested wheat seeds and attacking rice and soybean seeds in soil at a moisture level much below that required for the germination of the seeds.

Age of host plant. In general, southern blight is most destructive to young plants, although larger plants are often attacked and killed. Eight crops were planted and small lots of each of the eight were inoculated at

regular intervals throughout their period of growth. Six of the eight crops were highly susceptible to attack while in the seedling stage but became more resistant as they grew older. Tomatoes, cowpeas, and cotton became so resistant that later inoculations were almost wholly ineffective. Tomatoes remained susceptible longer than did the other two crops. Soybeans, field beans, and potatoes remained moderately susceptible throughout their growth period. Garden peas were highly resistant even in the seedling stage. Sugar beets remained highly susceptible throughout the season. Carrots and garden beets, though not included in these tests, were also highly susceptible late in the season. Thus it appeared that those crops with woody stems and roots became much less susceptible after the plants passed the herbaceous seedling stage, and only those with fleshy roots remained highly susceptible throughout the season.

Host range. The seedlings of 36 crops were tested for susceptibility to *S. rolfsii* in the greenhouse. The routine procedure entailed the use of 12 pots with four plants per pot for each crop. Two rooted cuttings of sweet potato were set in each of the 12 pots. Plants in six of the pots were inoculated with the B strain and the others with the R strain. All plants were inoculated while in the seedling stage. On the basis of the number of plants killed the crops were divided into two susceptibility groups: 1) Susceptible—garden beet, sugar beet, field bean, Lima bean, kidney bean, snap bean, cabbage, cotton, cowpea, cucumber, mustard, pepper, pumpkin, tomato, squash, radish, soybean, carrot, alfalfa, muskmelon, red clover, potato, turnip, Korean lespedeza, and tobacco. 2) Resistant—sweet potato, oat, peanut, garden pea, sweet corn, field corn, barley, sorghum, rye, wheat, and rice.

The resistant group included the various grasses, garden pea, peanut, and sweet potato. The fungus often grew vigorously around the stem of these resistant plants with no apparent injury to them. In general the seedlings of all the susceptible crops were killed when a sufficiently vigorous culture of the pathogen was used. There were no significant differences between the two strains of the pathogen on any of the 36 crops.

SURVIVAL OF SCLEROTIUM ROLFSII IN THE SOIL

Overwintering. Several field plots at Frederick, Maryland, were heavily infested with *S. rolfsii* in the summer of 1944. The infestation was sufficiently heavy to destroy more than 80 per cent of sugar beets and potatoes in the 1944 crop season. In the spring of 1945 various susceptible crops were planted over the 1944 plot area. No evidence of southern blight could be found on any of the crops. Samples of soil were taken into the laboratory at intervals during the spring and a search made for viable sclerotia. The method described by Leach (11) was used. Most of the soil samples contained no sclerotia and only two of the few sclerotia that were found could be germinated. This indicates that the winter of 1944–

1945 at Frederick was sufficiently cold for effective eradication of the pathogen from the soil.

Survival during one crop season. *S. rolfsii* survived in an active condition in greenhouse soil for only a few weeks. A soil bed in the greenhouse was heavily infested with *S. rolfsii*. Seeds planted immediately after soil infestation were rapidly destroyed by the fungus. Three weeks later seeds were planted and young tomato plants were set into the bed. No evidence of infection was observed on the seeds or on the plants. Sclerotia were in the soil in large numbers, but appeared in a dormant state. When removed from the soil, washed, and planted on agar, however, they grew as rapidly as sclerotia produced on laboratory media.

In subsequent greenhouse and field tests the active population of *S. rolfsii* in fallow soil dropped so rapidly that cowpeas and soybeans planted in the soil 2 weeks after soil infestation suffered little or no infection. When the infested wheat seeds were first added to the soil, the rapidly growing fungus quickly attacked and killed the seeds and susceptible plants. Within a week after infestation, however, sclerotia began to form and the mycelium gradually disappeared. *S. rolfsii* survived in an active state in the soil only when it was supplied with a suitable substrate such as the fleshy roots of beet or carrot or fruits such as melons or tomatoes. The cause of the apparent inactivation of the pathogen in the soil was not determined, but it is probable that other fungi, normally present in the soil, served to keep in check the population of *S. rolfsii* or that it was held in check by accumulations of its own metabolic products.

VARIABILITY WITHIN THE SPECIES SCLEROTIUM ROLFSII

In most pathogenic fungi, variation within a species is extremely common. *S. rolfsii*, however, has generally been reported to be fairly stable and uniform. Taubenhaus (26) and Weber (28) made comparisons of the morphological characteristics and host range of many isolates of *S. rolfsii* from different host plants and concluded that few or no strain differences occurred within the group of isolates studied. Nakata (19), Weber (28), and Takahashi (25) reported differences in size of sclerotia and in other morphological and physiological characteristics such as shape and color of sclerotia and type of growth in culture, and in reactions to nature of medium. No reports of clear differences in host range or relative pathogenicity of isolates have been noted.

In culture, the four strains used in these tests appeared almost identical until sclerotia began to form. The R strain normally produced a slightly heavier mycelial mat than any of the other strains. Sclerotia of the R strain were regularly larger and could be readily identified by this characteristic. The other three strains were morphologically indistinguishable. Sclerotial size varied somewhat with the medium on which they were produced, but those of the R strain were always larger on any given medium.

Average weights of 300 air-dried sclerotia produced on a cornmeal-peanut hull medium indicated that those of the R strain averaged 0.46 mg. in weight and 1.0 mm. in diameter; whereas those of the B strain averaged 0.33 mg. in weight and between 0.8 and 0.9 mm. in diameter.

Nakata (19) stated that *S. rolfsii* is a group species comprising numerous biologic forms which can be distinguished by the phenomenon of aversion. This phenomenon was described in earlier papers by Nakata (17, 18). Aversion was reported to occur between different strains of the fungus but not between two cultures of the same strain. When two colonies of the same strain are started at opposite sides of an agar plate, the colonies grow together and sclerotia are produced usually along a line where the colonies meet. When two colonies of different strains are so started and the colonies meet, the sclerotia are produced in two parallel bands leaving a narrow but distinct strip between the two lines of sclerotia (Fig. 2). The four strains

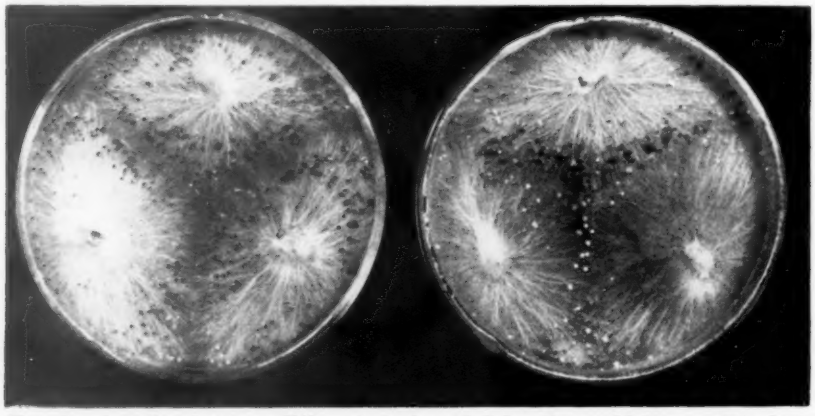

FIG. 2. Aversion between different strains of *Sclerotium rolfsii* on potato-dextrose agar. Left: One colony each of the B, R, and G strains showing distinct aversion. Right: Three colonies of the B strain showing no aversion.

of *S. rolfsii* included in these tests all showed a distinct aversion to each other, indicating that according to the concept of Nakata they all represented different and distinct strains. Reisolates after plant passage never showed aversion to the parent strain.

Taubenhaus (26) and Weber (28) isolated cultures of *S. rolfsii* from a number of different crops, and cross inoculated from one crop to another. Their results indicated that the original cultures varied only slightly in their virulence on any given crop regardless of the plant from which the cultures were originally isolated. No significant differences among isolates were indicated. All the four strains used in this study were tested on a variety of crops and no crop was susceptible to one strain and resistant to another. The relative degree of susceptibility of the crops to the four strains varied only slightly; all four were capable of killing essentially all the susceptible plants if vigorous inoculum was used. There were measurable and significant differences, however, in the rapidity with which the various strains

killed young plants. Differences in vigor of growth between cultures within any one strain were often considerable and rendered rather difficult accurate comparisons between strains. Therefore several cultures of each strain were used in each comparison, and a large number of comparisons were completed before definite conclusions were drawn.

The results in table 3 indicate that strain G was capable of killing

TABLE 3.—*Comparison of virulence of four strains of Sclerotium rolfsii on various crops in the seedling stage*

Crop	Average number of days elapsing between inoculation and death of plants			
	Strain G	Strain S	Strain R	Strain B
Test 1[a]				
Sugar beet	2.7	3.5	3.0	4.0
Cabbage	2.3	3.0	3.4	2.5
Muskmelon	2.3	3.1	2.7	3.3
Carrot	2.5	5.0	5.2	5.2
Cotton	2.0	2.2	2.2	2.8
Cowpea	4.1	4.3	4.5	4.9
Kidney bean	4.3	4.6	4.8	5.1
Radish	2.0	2.0	2.3	2.5
Soybean	3.1	2.9	3.1	3.6
Snap bean	3.0	3.1	3.3	3.6
Tomato	2.1	2.7	2.3	3.0
Potato	5.0	4.9	5.3	8.8
Average	2.94	3.45	3.52	4.10
L.D. (19: 1) between strain averages				0.40
Test 2[b]				
Cowpea	3.6	3.6	4.0	3.8
Tomato	2.1	2.1	2.6	2.2
Soybean	3.0	3.1	4.9	3.4
Radish	1.2	1.3	1.4	1.5
Average	2.46	2.54	3.23	2.69
L.D. (19: 1) between strain averages				0.17

[a] Twenty plants inoculated with each of two lots of infested seeds, or 40 plants per strain.

[b] Twenty plants inoculated with each of four lots of infested seeds, or 80 plants per strain.

plants more rapidly than were any of the other strains. Strain S killed plants somewhat more rapidly than did either R or B. These differences, though relatively small, were consistent and were statistically significant in most tests, and they undoubtedly represent true differences in the cultures. Usually the B strain was slightly more virulent than the R strain, but the difference was inconsistent and occasionally in the other direction. These differences were not correlated with the vigor of growth of the pathogen from the infested seeds. It is possible that the maintenance of cultures of the R and B strains on artificial media for several years had resulted in some degree of loss in virulence. The G strain was freshly isolated during the course of these studies and therefore had been in culture for only a short time. Taubenhaus (26) reported that old cultures were not pathogenic to plants but regained their virulence completely upon being trans-

ferred to fresh media. His methods were not sufficiently sensitive to detect the type of differences reported herein.

Only preliminary tests were conducted to determine the relative response of the various strains to different levels of soil temperature. The results indicated that all four strains reacted alike to temperature changes. The G strain was slightly more virulent as measured by rapidity of kill of seedlings at all five temperatures used in the tests, but its activity decreased at the lower temperatures as did the activity of the other three strains.

Saltation within the species *S. rolfsii* and within related genera and species has been observed only rarely. Nakata (20) reported the occurrence of saltants in only two of his cultures. Many thousands of cultures of four strains of *S. rolfsii* were made on many different types of media at Camp Detrick and no saltants were observed.

PERFECT STAGE OF SCLEROTIUM ROLFSII

According to Goto (7), the fungus *Corticium rolfsii* (Sacc.) Curzi is probably heterothallic, and natural or artificial crossing is very likely to occur. Other investigators (1, 16) state that the fungus is homothallic and that the perfect stage can be produced readily on onion agar or other special media.

Cultures of *S. rolfsii* of the four available strains were started August 15, 1944, on onion-extract agar. On September 13 half the cultures of each strain were exposed to direct rays of the sun for 3 hr. On September 27 white isolated islands of hymenial tissue were apparent in four of the plates which had been exposed to the rays of the sun, two of the S strain and two of the R strain. Microscopic examination of the tissue revealed immature basidia, but no spores. On November 24 basidia and apparently immature spores were observed, and on December 15 some apparently mature spores were found. Some of the basidial tissue was removed and placed in sterile water and the spores shaken free and cultured on clear agar. The few spores obtained and observed failed to germinate and no cultures were obtained. The fungus was shown to be homothallic since the perfect stage was obtained from a single culture, which, presumably, was from a single clonal line.

SUMMARY

The mycelium of *S. rolfsii* in air-dry, infested wheat seeds survived for at least a year with little apparent loss in viability when stored under cool (7°–8° C.), dry (12–25 per cent R.H.) conditions, but rapidly lost viability when stored under warm, humid conditions.

S. rolfsii was most active in soil at 30° to 35° C. Its activity was gradually reduced as the temperature was reduced until, at 15° C. or below, little activity occurred.

The mycelium of *S. rolfsii* was capable of growing and attacking seeds

in a soil with a moisture content much lower than that required for the germination of the seeds.

Of eight host plants tested throughout their period of growth seven were highly susceptible to attack by *S. rolfsii* in the seedling stage. The plants with woody stems, such as cotton, cowpea, soybean, and tomato, quickly became more resistant as they passed the seedling stage. Only the fleshy roots of sugar beets and similar crops remained highly susceptible to attack throughout the season. The pea was highly resistant to attack even in the seedling stage.

Thirty-six crops were tested for resistance to *S. rolfsii* in the seedling stage only. All the grasses tested were resistant. The dicotyledonous plants tested were all susceptible except pea, peanut, and sweet potato.

S. rolfsii failed to survive the winter of 1944–1945 in the soil in field plots at Frederick, Maryland, in sufficient quantity to cause visible damage to crops planted in the same plots the following summer.

S. rolfsii was capable of surviving in an active condition in a fallow soil either in the field or in the greenhouse for less than 2 weeks after the soil was artificially infested. It survived in an active state for the entire season only when such substrate as the fleshy roots of sugar beets or carrots was available.

Four strains of *S. rolfsii* were compared. The four were readily distinguished by their aversion to each other in agar plate cultures. They differed only slightly, but consistently, in virulence as measured by the rapidity with which they were able to kill young plants. One of the four was morphologically distinct and could be identified readily by its larger sclerotia. The other three were morphologically similar.

The perfect or *Corticium* stage of the fungus was produced on onion-extract agar in Petri-dish culture. Few basidiospores were produced, however, and none of these was germinated.

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RESISTANCE IN TOMATO TO THE SPOTTED-WILT VIRUS

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The resistance of the Red Currant tomato (*Lycopersicon pimpinellifolium*) to the spotted-wilt virus was reported by Samuel, Bald, and Pittman (10) in 1932 and subsequently by others (2, 3, 11). Resistance has also been reported in *L. hirsutum* (3), another species which will hybridize with the cultivated tomato, but, as with Red Currant (11), not all strains are resistant. *L. peruvianum* is highly resistant (11) but its cross-sterility with the cultivated tomato has prevented its use. *L. glandulosum* appears to have some degree of resistance.

Certain varieties of the cultivated tomato have been reported to be resistant (4, 9, 10). Three of Holmes' (4) resistant selections proved highly susceptible in our plots. The German Sugar variety has marked resistance (7, 11).

Dr. D. R. Porter early recognized the disease-resistance potentialities of the Red Currant tomato. Using a strain that was highly resistant to spotted wilt, he made certain crosses with commercial varieties which eventually yielded the resistant hybrid used by Kikuta and Frazier (6, 7) as the source of resistance in their new variety, Pearl Harbor. Porter's strain of Red Currant has also been used as a source of resistance by Hutton and Peak (5).

The present report is on the resistance of Porter's strain of Red Currant, the German Sugar variety, and hybrids between them and commercial varieties. Resistance was determined by exposure of replicated lots of 20 plants each to natural infection in field plots located in districts where experience indicated that infection was likely to occur. There was great variation in percentage of infection from year to year and between localities, depending on movement and infectivity of the thrips population. Apparently most of the infection of tomatoes is brought about by infective thrips moving in from outside sources of infection, as observed by Bald (1). There does not appear to be much spread from plant to plant in a tomato field.

Field plots were located in California at San Pablo for 12 years (1936 to 1949), and also at Mt. Eden the first 6 years and at Salinas the last 8 years. Heavy infection occurred at San Pablo except in 1940 to 1943 and 1945, at Mt. Eden in 1936, and at Salinas in 1942, 1943, and 1947. Disease counts were made at frequent intervals. The leaf symptoms and tip blight developed when the plants were infected while in active vegetative growth, whereas with late infection fruit rings were often the only symptoms available for diagnosis.

RESISTANCE OF RED CURRANT HYBRIDS

The high resistance of Porter's strain of Red Currant, as pointed out in 1944 (11), has continued in our subsequent plot tests. With the exception of local lesions no symptoms of spotted wilt have been found on this strain in the field although it is susceptible to artificial inoculation (5, 8).

Selections for resistance were made by Porter in 1936 and 1937 from his crosses between Red Currant and a number of commercial varieties including a green-wrap type, 133–6, derived from the Early Santa Clara variety. In 1939, Dr. G. N. Davis selected from among the F_4 of one of Porter's back crosses, 133–6 × (133–6 × Red Currant), one line (BC–10) having a high level of resistance under conditions of heavy infection. BC–10 was later crossed with the Bounty variety in Hawaii by Kikuta and Frazier (6, 7), who thus derived the resistant Pearl Harbor lines, many of which have been included in our plots since 1944.

In the 1939 San Pablo plot referred to above, BC–10 remained free of infection, whereas 30 similar lots in the F_3 or higher generations derived from the first backcross to 133–6, or the first and second outcrosses to commercial varieties, had 6 to 100 per cent infection. Two closely related sib lines had 30 per cent and 100 per cent infection. However, in 1942 and 1943 at Salinas and 1947 and 1948 at San Pablo it was found that BC–10

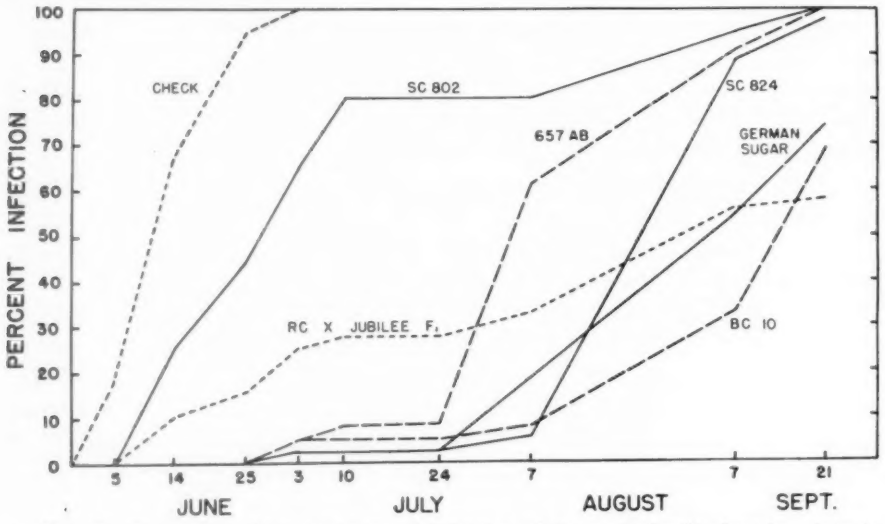

FIG. 1. Seasonal incidence of natural infection of the spotted-wilt virus in a tomato field plot, San Pablo, California, 1948. Resistant lines escape early infection. Red Currant (not shown) remained free from infection. BC–10 resulted from a cross between Red Currant and 133–6 backcrossed to 133–6. 657AB is a Pearl Harbor line with resistance derived from BC–10. SC824 is 657AB × (German Sugar × 133–6 F_2) combining resistance from two sources. SC802 is a backcross of the same German Sugar × 133–6 F_2 plant to its susceptible parent, 133–6.

was susceptible to late season infection (Fig. 1) and does not possess the same level of resistance as the Red Currant parent.

When the checks become infected early in the season and bear little or

no fruit, BC–10 and the Pearl Harbor lines tend to escape early infection, have relatively few plants with foliage symptoms, and set a crop of fruit. Late in the season, however, the circular spots and rings characteristic of spotted wilt appear rather generally on the fruits of these hybrids. From the grower's standpoint, the tendency to escape early infection is important.

A backcross of BC–10 to its susceptible parent 133–6 proved definitely less resistant than BC–10 under heavy infection in 1948.

An F_1 of Jubilee (susceptible) × Red Currant had an intermediate degree of resistance in 1948 (Fig. 1), and F_2 lines behaved similarly in 1949. Samuel, Bald, and Pittman (10) found the F_1 of Red Currant hybrids susceptible. Costa (3) reported good resistance in hybrids of Red Currant × Early Jewel.

The possibility of increasing resistance by selection within the more resistant breeding lots appears remote. Year after year of selection of seed from apparently healthy surviving plants among the Pearl Harbor and other hybrids has not altered the degree or type of resistance.

The Red Currant level of resistance has not been recovered in any of its progeny. With each backcross to the susceptible type, a certain amount of resistance appears to be lost. Inheritance of resistance is apparently due to multiple factors, as indicated by lack of clear-cut levels of resistance and the intermediate level of resistance of the F_1 and F_2 generations.

RESISTANCE OF GERMAN SUGAR HYBRIDS

In 1947 the F_2 progenies of German Sugar crossed with the varieties 133–6, Earliana, Pearson, and Best-of-all had a rate of infection intermediate between that of German Sugar (40 per cent) and the checks (100 per cent), with final percentages of 87 to 95. These hybrids and the German Sugar parent, like the Red Currant hybrids, also tended to escape early-season infection. The F_2 progenies were somewhat less resistant than the F_1. Selection to the F_3 did not increase resistance.

Backcrosses of these hybrids to German Sugar retained a resistance about equal to that of the F_1. Backcrosses to their susceptible parents resulted in loss of resistance (Fig. 1).

Cuttings made from a few of the German Sugar × 133–6 F_2 hybrids that survived in 1947 were grown in the greenhouse and crossed with one of the Pearl Harbor lines (657AB), thus combining resistance from two sources. In 1948 and 1949 these lines had a degree and type of resistance similar to that of Pearl Harbor (Fig. 1).

SUMMARY

Resistance of tomato hybrids to natural infection of the spotted-wilt virus has been tested in field plots during 12 years.

Porter's strain of the Red Currant tomato has consistently escaped infection. The German Sugar variety is moderately resistant.

Hybrids of Red Currant and standard varieties have yielded lines in-

cluding the Pearl Harbor types with an intermediate level of resistance, characterized by a tendency to escape early infection and bear a crop despite late infection.

The Red Currant level of resistance has not been recovered in any of its progeny. Evidence of simple Mendelian inheritance of resistance has not been obtained.

Hybrids of German Sugar and standard varieties likewise have shown an intermediate level of resistance.

Combination of resistance from Red Currant and German Sugar has not raised the level of resistance.

Backcrosses to the susceptible parent have resulted in loss of resistance.

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INFECTION IN SEEDS AND SEEDLINGS OF WHEAT AND BARLEY IN RELATION TO DEVELOPMENT OF LOOSE SMUT[1]

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Loose smut of wheat (*Ustilago tritici* (Pers.) Rostr.) and loose smut of barley (*U. nuda* (Jens.) Rostr.) are carried over from one season to another by means of dormant mycelium within the seeds of their respective hosts. On germination of an infected kernel, the mycelium begins to grow, invades the seedling tissues, and eventually produces spores in the inflorescence and, occasionally, in the leaves of the adult plant.

In infected seeds of susceptible wheat and barley, the loose-smut mycelium is found in the embryos (1, 2, 5, 6, 7, 8, 11, 12, 13, 14, 16, 18, 19, 20, 21). In resistant varieties of wheat (5, 14, 16, 21) and in one resistant variety of barley (16) the embryos were not infected except for a small minority in one variety of wheat (5). In certain susceptible varieties of wheat the infected embryos were often sparsely invaded with mycelium, and partially infected plants and heads were frequent (14).

Examination of considerable numbers of seeds or seedlings for loose-smut mycelium is practicable only when a reasonably rapid method of detection is used. In various studies hyphae in seeds of wheat grew out into culture media and thus indicated the presence of infection (3, 4); infected seedlings of wheat were detected by visual inspection of the young developing heads about a month after the seed had seen sown (17); entire embryos of wheat and barley were extracted from seeds and examined for infection (19, 20); and mycelium was detected in unsectioned tissue of wheat seedlings (18) and in sectioned and stained wheat and barley embryos (9, 10, 19, 22) by means of modified techniques.

Very few comparisons have been made, as yet, between the incidence of infection in embryos or in seedlings and that of smut in adult plants. In two varieties of barley and one variety of wheat examined, the percentage of infected embryos corresponded closely to the percentage of smutted plants (19).

The usual method of estimating loose-smut infection in seed of wheat and barley is to sow the seed in the greenhouse or the field and record the percentage of smutted plants. If, however, the percentage of smutted plants could be determined in advance by examining embryos or seedlings for smut mycelium, the investigation of certain aspects of loose-smut infection (e.g., the determination of varietal resistance or the need for treating seed for loose-smut control) would be greatly facilitated. For the success-

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ful application of the embryo or seedling method of diagnosis, it would be necessary to have a rapid and reliable technique for detecting the presence of loose-smut mycelium, and adequate experimental data on the relationship between infection in seeds or seedlings and the presence of smut in adult plants.

The investigations reported in this paper were undertaken 1) to improve existing methods of examining seeds and seedlings of wheat and barley for the presence of loose-smut mycelium, and 2) to ascertain to what extent infection in seeds and seedlings is correlated with smut in adult plants of susceptible and resistant varieties.

MATERIALS AND METHODS

Unless otherwise stated, the seed for experimental purposes was collected from wheat and barley varieties that had been artificially inoculated with loose smut by the method described by Moore (15). As a variety of wheat or barley may be susceptible to one physiologic race of loose smut and resistant to another, each variety investigated was inoculated with at least two different races. A single variety was studied, therefore, both as a susceptible and as a resistant variety. Seed lots were divided into different parts, each part being used for a study of infection in a different stage of plant development.

Before seed embryos could be prepared for examination, it was necessary to separate them from the endosperm and pericarp. In a modification of Skvortzov's technique (20), kernels were macerated for 48 hr. in a 10 per cent solution of potassium hydroxide at room temperature. During maceration, the endosperm and embryo became swollen and usually ruptured the pericarp. The solution and macerated seed were poured into a 10-mesh sieve, through which the embryos were strained into a beaker with the aid of a rinse spray. The solution was then poured off and replaced with a 10 per cent solution of potassium hydroxide, and in it the embryos were boiled for about 1 min. to clean their surfaces. Finally, the embryos were washed twice in boiling water for about 1 min. each time to leach out most of the potassium hydroxide.

To facilitate the detection of mycelium, the embryos were boiled for about 4 min. in a staining solution.[3] The treated embryos were mounted in rows on glass slides and slightly flattened under a cover glass for examination under the microscope. The mounting medium was a solution of 1 part glycerin and 2 parts distilled water. During treatment, the embryo tissue became light blue and translucent. The mycelium was more darkly stained and could be recognized easily in the semitransparent host tissue. An embryo was considered to be infected if smut mycelium was found in any part of it.

[3] Lactic acid, 12.5 cc.; phenol (at 45–50° C.), 12.5 cc.; glycerin, 12.5 cc.; distilled water, 62.5 cc.; and Poirrier's blue (cotton blue), 0.01 gm. In making up the solution, the cotton blue was dissolved in lactic acid and water, and the other ingredients were then added.

Seedlings of wheat and barley examined for loose-smut mycelium were grown in the greenhouse from seed treated with an organic-mercury dust in order to prevent infection by surface-borne smuts. The seed was sown 1 in. deep in pots of soil, and the pots were placed in subdued light. Under these conditions, the seedlings developed fairly long subcrown internodes, and the tissue could be easily excised without including a portion of the scutellum. A portion of each seedling, including the crown node, about $\frac{1}{8}$ in. of the subcrown internode, and $\frac{3}{8}$ in. of the shoot (plumule), was excised for examination of the growing point after the tip of the second leaf had made its appearance. Comparable pieces of tissue excised from wheat seedlings in about the six-leaf stage (shortly before the "shooting" period) were used for a study of infection in young developing heads. First the coleoptile and, in old seedlings, some of the leaf tissue were stripped off and the excised pieces were boiled in a 10 per cent solution of potassium hydroxide for about 5 min. or until the tissue became semitransparent. The seedling pieces were then washed, stained, and mounted in the same manner as the embryos.

The scutellum attached to the seminal-root node of plants was also excised and examined for mycelium, as it does not easily lose its identity with the caryopsis during plant development. The method of preparing the scutellum for examination was the same as that used in the study of growing-point tissue of seedlings. By examination of the scutellum, it was possible to determine whether or not infection was present in the embryo from which the plant originated, and to obtain supplementary evidence on the relationship between the incidence of infection in embryos and plants. The data on scutellum infection also served to show whether or not the incidence of infection in seed embryos from which plants were actually grown was similar to that in the embryos examined for infection.

The possibility of confusion in the interpretation of results owing to the presence of other fungi among the hyphae of loose smut of wheat or loose smut of barley was not an important consideration in this study. Such fungi were rarely in evidence in seed embryos and not at all in meristematic tissue of seedlings, but were frequent in or near the surface tissue of the scutellum of plants. They were usually inconspicuous, however, as they rarely became stained. The hyphae of loose smut of wheat and loose smut of barley were readily identified in embryos and seedling tissues; they were usually short, irregularly swollen, and thick walled, and stained readily.

Unless otherwise stated, percentages in the tables are based on examinations of 100 seeds for embryo infection, 200 seedlings in the two-leaf stage for scutellar and growing-point infection, 200 seedlings in about the six-leaf stage for infection of young developing heads, and 200 adult plants for smutted heads. Fractional percentages of 0.5 are recorded as 1 per cent.

Simple correlation coefficients were calculated to determine the extent of agreement between infection readings obtained by examining embryos, scutella, growing points, and adult plants. In experiments (Table 1, A,

race C; Table 3, races D and E; and Table 5, race C) where the percentages of infection are not distributed normally because of the considerable number of zero readings, no correlation coefficients are presented.

Where varieties of wheat or barley are described as "susceptible" or "resistant" to loose smut and not otherwise qualified, the terms refer to the reactions of the adult plants.

ARTIFICIALLY AND NATURALLY INOCULATED WHEAT

In this experiment, a study was made of the percentage of smutted plants in relation to the percentage of seed embryos and seedlings showing the presence of mycelium. Eight varieties of wheat were used, each one inoculated separately with two races of loose smut. The results are summarized in table 1, A.

The relationship between the incidence of infection in seed embryos and that in adult plants (Table 1, A) varied widely in different varieties inocu-

TABLE 1.—*Comparison of infection in seeds, seedlings, and adult plants of wheat inoculated artificially and naturally with loose smut*

Variety	Race	Percentage infection			
		Seeds (embryos)	Seedlings		Adult plants
			Scutellum	Growing point	
A. Inoculated artificially					
Reward	A	90	92	92	94
Do	C	37	34	2	1
Renfrew	A	56	60	31	29
Do	C	0	0	0	0
Mindum	A	77	79	0	0
Do	C	87	92	92	90
Marquis	A	69	70	65	66
Do	C	0	0	0	0
Garnet	A	96	92	92	98
Do	C	21	17	0	0
Thatcher	A	75	80	6	4
Do	C	0	0	0	0
Regent	A	89	80	27	32
Do	C	0	0	0	0
Renown	A	79	82	41[a]	4
Do	C	0	0	0	0
B. Inoculated naturally					
Reward		17	17	12	11
Renfrew		4	5	2	2
Mindum		8	9	4	5
Marquis		10	11	11	11
Garnet		15	13	10	9
Thatcher		9	8	0	0
Regent		13	11	5	4
Renown		12	11	4[b]	0

[a] In most growing points the mycelium was sparse.
[b] In all growing points the mycelium was sparse.

lated with either race of smut. The percentage infection in seeds and adult plants agreed fairly closely in three varieties susceptible to race A, in one variety susceptible to race C, and in five varieties immune from race C.

In the remainder of the experiment, infection was prevalent in embryos but either much less prevalent or lacking in adult plants. For the experiment as a whole, the average percentage infection for embryos was 50, and for adult plants 26. These results are substantiated by the fact that mycelium was often found in the scutellum of smut-free plants, thus indicating that smut-free plants may sometimes be produced from infected seeds (embryos).

The seedling (growing point) reaction corresponded closely to the adult-plant reaction in all varieties except Renown (inoculated with race A). Even in this variety adult-plant reaction (4 per cent) was in closer agreement with seedling (41 per cent) than with seed-embryo reaction (79 per cent). It should be pointed out, however, that the majority of the infected seedlings of Renown were sparsely invaded with mycelium.

These results suggest that the immunity or high degree of resistance possessed by certain varieties of wheat results from inability of the loose-smut mycelium to invade the embryo. In other varieties, in which a high proportion of the embryos become infected, resistance seems to be due to the inability of the mycelium to invade the growing point, or to its failure to survive in the plant after invasion from the embryo has taken place.

The percentages of infection in seeds, seedlings, and adult plants were also determined in naturally inoculated wheat. The seed used in this experiment was collected from plots of eight varieties of wheat grown from seed of plants that had been inoculated artificially with spores of race A or C. The results are in table 1, B.

In five of the eight varieties of wheat, there is fair agreement between the percentage of infected embryos and the percentage of smutted plants. In the experiment as a whole, however, the percentage of smutted plants is more closely related to seedling (growing point) infection than to embryo infection. The reaction of Renown merits special comment in that it was infected in the seedling stage and yet failed to produce any smutted plants. Mycelium was sparse in infected seedlings of this variety and this condition may be related to the absence of smutted heads in adult plants.

MYCELIUM IN YOUNG AND OLD SEEDLINGS OF RENOWN AND REWARD WHEAT IN RELATION TO THE INCIDENCE OF SMUT IN ADULT PLANTS

In view of the discrepancy between the seedling and adult-plant reactions in Renown wheat, it seemed possible that the abnormal type of seedling infection observed in this variety might be due to a lack of compatibility between host and parasite, resulting in suppression of smut development in the inflorescence. To determine at what stage of seedling development the decline in infection occurred, it was decided to investigate the incidence of mycelial invasion in both young and older seedlings of Renown in relation to the incidence of smut in adult plants.

Renown and Reward wheat, inoculated with race A, were used in this experiment, as the former variety is resistant and the latter susceptible to this race. Seedlings were examined in the two-leaf stage for mycelium in

the region of the growing point, and in the six-leaf stage (shortly before the "shooting" period) for mycelium in the young developing head.

Percentages of infection in young and old seedlings and adult plants were very similar in Reward wheat (96, 93, and 97 respectively), but widely dissimilar in Renown. In the latter variety, the infection in old seedlings (20 per cent) was closer to that in adult plants (5 per cent) than the infection in young seedlings (49 per cent).

The mycelium in most seedlings of Renown was sparse in comparison with that in Reward and the hyphae in Renown seedlings often appeared degenerate. Some additional observations indicated that the percentage of seedlings that were normally or nearly normally invaded with mycelium agreed closely with the percentage of smutted plants. Thus, the more abundantly invaded seedlings were indicative of the development of smut in adult plants, and those less abundantly and sparsely invaded were indicative of smut-free plants.

With further reference to the relationship between heavily invaded seedlings and smut in adult plants of Renown wheat, it should be pointed out that, when the plants tillered, not all of the heads of individual plants were smutted and the affected heads themselves were usually partially smutted. This condition appeared to be influenced by the distribution of mycelium in the tissues of the seedlings. In young seedlings, mycelium was not present in the vicinity of all developing shoots of individual plants and, in older seedlings, the tissue of young developing heads was noninvaded or only partially invaded with mycelium.

INFECTION IN SEEDS AND ADULT PLANTS OF WHEAT

In previous experiments, it was evident that infected embryos were sometimes prevalent in varieties of wheat that produced little or no smut in adult plants. According to the available literature, the embryos of resistant varieties are rarely or not at all invaded by the loose-smut fungus. To obtain supplementary information on this question, a study was made of the percentages of infection in seeds and adult plants of six varieties of

TABLE 2.—*Comparison of seed and adult-plant infection in wheat inoculated artificially with loose smut in three different years*

Variety	Percentage infection							
	1939		1940		1941		Average	
	Embryos	Adult plants	Embryos	Adult plants	Embryos	Adult plants	Embryos	Adult plants
Reward	95	96	91	88	83	86	90	90
Marquis	79	76	63	51	69	63	70	63
Garnet	27	23	62	58	68	64	52	48
Apex	41	23	18	10	35	13	31	15
Renown	76	4	82	7	82	6	80	6
Thatcher	45	0	41	1	53	2	46	1

wheat, including the resistant varieties Apex, Renown, and Thatcher, inoculated with race A of loose smut in 1939, 1940, and 1941.

The results (Table 2) show that the percentage of infected seeds equalled or nearly equalled the percentage of smutted plants in three varieties, and greatly exceeded it in the remaining varieties. The relative amounts of infection in seeds and adult plants were fairly constant in different years. These results confirm those obtained earlier, and show that smut infection may often be prevalent in seed, although much less prevalent or absent in adult plants grown from it.

As there was evidence (Table 1, A) that the relative percentages of infection in seed embryos and adult plants of wheat are influenced by the race of loose smut used for inoculum, further experiments were conducted with other races. The results, based on 10 varieties of wheat artificially inoculated with spores of three races, are in table 3.

TABLE 3.—*Comparison of embryo and adult-plant infection in wheat inoculated artificially with different races of loose smut*

Variety	Percentage infection					
	Race B		Race D		Race E	
	Embryos	Adult plants	Embryos	Adult plants	Embryos	Adult plants
Reward	91	96	55	2	95	3
Renfrew	68	51	0	0	45	0
Mindum	46	0	41	42	0	0
Marquis	43	24	0	0	15	2
Garnet	56	49	15	0	66	1
Thatcher	42	0	0	0	15	0
Regent	86	16	0	0	66	1
Renown	55	1	0	0	27	0
Red Bobs	77	72	52	0	95	2
Pentad	50	0	87	86	38	0

The relation between the percentages of infection in seeds and adult plants varied in different varieties regardless of the race used for inoculum. Infected embryos were often prevalent in varieties that developed little or no smut in plants. Race D had a lesser tendency to infect the embryos of varieties with immunity or a high degree of resistance in the adult-plant stage than did the other races. In the experiment as a whole, infection averaged 44 per cent in seed embryos and 15 per cent in plants. These results are very similar to those obtained in previous experiments (Table 1).

As may be seen in tables 1, A, and 3, when experiments are limited to a few varieties infected with certain races, it is possible to obtain results indicating that infected embryos are prevalent in susceptible and not in resistant varieties. This factor may account for the reports of failure to find infected seed embryos in resistant varieties.

ABUNDANCE OF MYCELIAL INVASION IN WHEAT EMBRYOS IN RELATION TO SMUT IN PLANTS

Since the incidence of embryo infection in wheat was frequently much higher than that of adult-plant infection and there was much variation in the degree to which individual embryos of wheat were invaded with mycelium, a study was made of the relationship between the extent of mycelial invasion in the embryo and the occurrence of smut in the adult plant.

Eight varieties of wheat, artificially inoculated with spores of race A, were studied. On examination, embryos were classified on the basis of the amount of tissue invaded with mycelium. An arbitrary scale (1 to 5) was adopted in order to place the infection ratings on a numerical basis. The scale was divided as follows as to degree of infection: 1 indicates trace of mycelium, and 2, 3, 4, and 5 indicate about one-fourth, one-half, three-fourths, and the whole of the embryo, respectively, invaded with mycelium. The results of the examinations are in table 4, together with the percentages of smut in adult plants grown from the same lots of seed.

TABLE 4.—*Comparison of the extent of embryo invasion and adult-plant infection in wheat inoculated artificially with race A of loose smut; 200 embryos and 200 adult plants examined*

Variety	Percentage infection						
	Embryos—extent of invasion[a]						Adult plants (smutted)
	1	2	3	4	5	1–5	
Reward	0	0	4	2	84	90	94
Renfrew	4	8	17	13	18	60	29
Mindum	17	38	22	0	0	77	0
Marquis	0	6	18	14	29	67	58
Garnet	0	2	10	53	31	96	98
Thatcher	2	4	18	18	22	64	4
Regent	4	2	11	32	41	90	32
Renown	0	2	6	24	48	80	4

[a] Invasion index based on amount of embryo tissue invaded with mycelium: 1, trace of mycelium; 2, about one-fourth of embryo invaded; 3, about one-half of embryo invaded; 4, about three-fourths of embryo invaded; 5, nearly all of embryo invaded.

There is considerable variation in the amount of mycelium in embryos of resistant and susceptible wheats, and embryos of resistant varieties may be as heavily invaded as those of susceptible varieties. In susceptible varieties (Reward, Marquis, and Garnet) there was a close correlation between the percentage of moderately to heavily infected embryos (infection ratings 3 to 5 inclusive) and the percentage of smutted plants; but in the remaining varieties there was no correlation in this respect, as the moderately to heavily invaded embryos were usually very prevalent, whereas smutted plants were much less prevalent or even absent. It is concluded, therefore, that in susceptible varieties abundantly invaded seed embryos produce smutted plants, whereas in resistant varieties such embryos often produce smut-free plants. On the whole, the occurrence of smut in plants could be

TABLE 5.—*Comparison of infection in seeds, seedlings, and adult plants of barley inoculated artificially with loose smut*

Variety	Percentage infection											
	Race A				Race B				Race C			
	Seeds (embryos)	Seedlings		Adult plants	Seeds (embryos)	Seedlings		Adult plants	Seeds (embryos)	Seedlings		Adult plants
		Scutellum	Growing point			Scutellum	Growing point			Scutellum	Growing point	
O. A. C. 21	58	52	52	54	6	8	7	7	0	0	0	0
Trebi	16	15	10	11	55	53	53	58	6	4	4	5
White Hulless	40	37	37	39	4	3	2	2	0	0	0	0
Success	67	65	65	70	60	57	57	59	1	1	1	1
French Chevalier	90	87	87	87	71	73	73	71	69	73	73	72
July	33	30	30	27	1	1	1	1	2	2	2	1
Velvet	14	15	15	16	9	11	11	10	0	0	0	0
Hannchen	71	65	65	68	57	49	49	54	10	11	11	10
Garton's	47	48	48	50	34	37	37	35	0	0	0	0
Atlas	64	68	68	64	4	4	4	5	0	0	0	0
Plush	81	78	78	70	81	78	70	72	6	6	6	4
Mensury (Ott. 60)	81	77	77	77	21	16	16	17	0	0	0	0
Rex	75	76	76	70	78	79	79	75	0	0	0	0
Prospect	68	65	65	69	63	63	63	68	1	1	1	1
Newal	55	53	53	51	27	21	21	25	1	2	2	1
Olli	47	46	46	47	24	25	25	20	0	0	0	0
Colsess	52	53	53	53	54	53	53	59	0	0	0	0

predicted more closely when based on moderately to abundantly invaded embryos than on the total number infected.

The conclusions drawn with respect to the data presented in table 5 are further substantiated by information gained from the examination of the scutella of plants. Scutellar tissue of smutted plants was abundantly invaded with mycelium and that of smut-free plants was noninvaded, sparsely, or abundantly invaded, depending upon the plant examined. Sparsely invaded scutellar tissue was found in smut-free plants of susceptible as well as resistant varieties, thus indicating that susceptible plants may escape infection if embryos are lightly invaded with mycelium.

INFECTION IN SEEDS, SEEDLINGS, AND ADULT PLANTS OF ARTIFICIALLY AND NATURALLY INOCULATED BARLEY

Seventeen varieties of barley were examined to determine the incidence of smut infection in seeds and seedlings in relation to that in adult plants. This study was made with naturally inoculated seed as well as with seed that had been inoculated artificially with three races of loose smut, the varieties being inoculated separately with each race.

The results obtained from artificially inoculated barley (Table 5) show that the incidence of smut in both susceptible and resistant varieties agrees closely with the incidence of mycelium in seed embryos or seedlings. The investigation in which naturally inoculated seed was used yielded very similar results (Table 6). In some varieties small discrepancies occurred. In these varieties, there was evidence of the production of smut-free plants from sparsely infected embryos, as the scutellar tissue of such plants was lightly infected with mycelium.

TABLE 6.—*Comparison of infection in seeds, seedlings, and adult plants of barley inoculated naturally with loose smut*

Variety	Percentage infection[a]			
	Seeds (embryos)	Seedlings		Adult plants
		Scutellum	Growing point	
O. A. C. 21	25	23	18	16
Trebi	6	6	2	2
White Hulless	8	6	6	7
Success	9	10	9	11
French Chevalier	25	23	23	25
July	11	10	4	5
Velvet	1	2	0	0
Hannchen	15	12	12	13
Garton's	10	10	9	9
Atlas	12	10	7	8
Plush	22	26	26	23
Mensury Ott. 60	13	12	12	14
Rex	8	10	8	7
Prospect	9	11	11	11
Newal	13	12	10	10
Olli	5	6	4	4
Colsess	8	7	5	4

[a] Based on an examination of 200 embryos, 200 seedlings, and 200 adult plants.

TABLE 7.—*Correlation coefficients for percentage infection of wheat and barley as determined in seeds and plant tissues*

Crop and table (source of data)	Race of smut	Correlation coefficients (r)[a]						d / f	r at 5 per cent point
		Emb. Scut.	Emb. G. P.	Emb. A. P.	Scut. G. P.	Scut. A. P.	G. P. A. P.		
Wheat									
1	A	0.938	0.468	0.474	0.468	0.427	0.940	6	0.707
2	Natural inoculation	0.954	0.803	0.526	0.817	0.683	0.946	6	0.707
3	A (1939)			0.678				4	0.811
3	A (1940)			0.601				4	0.811
3	A (1941)			0.601				4	0.811
3	A (average 1939–1941)			0.574				4	0.811
4	B			0.262				8	0.632
5	A			0.556				6	0.707
5	A			0.665[b]				6	0.707
Barley									
6	A	0.993	0.992	0.985	0.999	0.986	0.988	15	0.482
6	B	0.994	0.991	0.992	0.998	0.991	0.993	15	0.482
7	Natural inoculation	0.961	0.915	0.907	0.965	0.929	0.981	15	0.482

[a] Abbreviations for names of tissues examined; Emb., embryo; Scut., seedling scutellum; G. P., seedling growing point; A. P., adult plant.
[b] Lightly infected embryos classed as noninfected.

COMPARISON OF INFECTION DATA OBTAINED FROM DIFFERENT METHODS OF EXAMINATION

Simple correlation coefficients calculated to determine the extent of agreement between infection readings obtained by examining embryos, scutella, growing points, and adult plants of wheat and barley varieties (Table 7) indicate that the percentage infection in adult plants of wheat is not highly correlated with that in embryos and scutella. When heavily infected embryos only are classed as infected, the correlation (embryo–adult plant) is slightly higher than when all infected embryos are included.

The readings on infection in growing points of wheat are highly correlated with those of smut in adult plants.

As might be expected, the correlation between percentage infection in embryo and in scutellum of wheat is high, as the embryo tissue is largely scutellum and the scutellar tissue of the seedling is the same as that in the embryo. The high correlation between embryo and scutellum is evidence of the homogeneity of the seed used for the determination of infection in embryos and growing points of seedlings.

As the correlation between percentage infection in growing point and adult plant of wheat is highly significant, whereas other correlations (embryo–adult plant and scutellum–adult plant) are not significant, it would seem to follow that the correlations (embryo–growing point and scutellum–growing point) would prove to be nonsignificant. The analysis shows, however, that the latter correlations are nonsignificant for data from A in table 1, but not for those from B. These correlations, however, are not so highly significant as those between growing point and adult plant.

The explanation for the disagreement between the correlations derived from data in table 1 may lie in the fact that the correlations for part A are based only on race A, whereas those for part B are based on naturally inoculated seed that might have been infected with several races. Whatever the explanation may be, it is evident that the results obtained with respect to the principal objective of the analysis, namely, the determination of infection in embryos and seedlings of wheat (growing points) in relation to the development of smut in the adult plant, are consistent in different experiments, and indicate that the incidence of smut in adult plants is highly correlated with that of infection in seedlings but not with that of infection in embryos.

In the barley experiments, all correlations are high and statistically significant, indicating that the various methods of predetermining smut infection in plants may be used interchangeably. Although no correlation coefficient is given for data from table 5 (race C) because of the large number of zero readings, it will be noted that the percentages of infection for embryos, scutella, growing points, and adult plants are in close agreement.

DISCUSSION

The two principal objectives of the investigations were to improve existing methods of detecting the presence of loose-smut mycelium in seeds and

seedlings of wheat and barley, and to ascertain the extent to which infection in seeds and seedlings is correlated with the incidence of smut in adult plants.

The methods employed were basically the same as those previously used by a number of other investigators. It was possible, however, to effect improvements by several modifications in technique. The methods finally adopted were sufficiently simple and rapid to permit the examination of a large amount of material. Furthermore, it is believed that they may be relied upon to reveal the presence or absence of loose-smut mycelium in tissues of embryos and seedlings of wheat and barley and, where mycelium is present, to indicate the extent of invasion.

The relationship between loose-smut infection in embryos and seedlings of wheat and that in adult plants is by no means a simple one. It is influenced by the wheat variety, the physiologic race of smut used as inoculum, the amount of mycelium present in individual seed embryos, and the interaction between host and parasite at different stages of plant development. The embryos of some wheat varieties are apparently immune from certain races of loose smut and susceptible to others. If immune, there is no smut mycelium in the embryos, and no smut will appear in adult plants developing from them. If the embryos are susceptible and become infected, smut may or may not appear in the inflorescences to which they give rise, depending upon the amount of mycelium present in individual embryos and the susceptibility or resistance of the variety during plant development. The interaction between variety and physiologic race of loose smut is clearly indicated by the fact that a variety, when inoculated with spores of a certain race, may become heavily infected in both the embryo and adult-plant stages, whereas, when inoculated with other races, it may either remain immune from infection in all stages of development or produce a high proportion of infected embryos but a lower proportion of smutted plants, or none at all. The particular type of reaction will depend upon the wheat variety and the race of smut used as inoculum. A similar relationship is apparent when several varieties are inoculated with spores of the same race of loose smut.

These observations suggest that, in certain varieties, a high proportion of the embryos may be infected but mycelium may be present in a much smaller proportion of the seedlings, and the infected seedlings themselves may or may not all develop into smutted plants.

The conclusion is that two factors are operative in determining the resistance of wheat varieties to loose smut. The first of these resides in the tissues of the embryo which, in certain varieties, apparently are able to prevent entirely the invasion of the smut mycelium or to inhibit the development of hyphae that may gain entrance. The second factor becomes operative in the later stages of plant development, extending from the seedling to the adult plant. Here the tissues of certain varieties appear to be able to suppress or inhibit the development of loose-smut mycelium, with the result that seeds which are known to have a high incidence of embryo infec-

tion may produce few or no smutted plants. Both factors seem to be present in varying degrees in most of the varieties of wheat used in these investigations. Whether the factors responsible for embryo and seedling resistance are physiological or morphological in nature remains to be determined.

Thus, loose-smut infection in adult wheat plants cannot be predicted with any degree of accuracy by examining embryos for the presence of mycelium or by classing only heavily invaded embryos as infected, although the latter method is more reliable than the former. A more reliable index of adult-plant infection might be expected from an examination of seedling tissues, but even when this method is used the percentage of infected seedlings may in some instances exceed the percentage of smutted plants.

The susceptibility or resistance of barley varieties to loose smut is fairly constant during the life cycle of the plant. Whether or not there are races of this smut to which some barley varieties are susceptible in the embryo stage and resistant in the seedling or adult-plant stages is not at present known. This phase of the problem requires further study if infection of seed or seedlings is to be used as an index of smut infection in crops.

SUMMARY

In a study of the relative amounts of infection in seed embryos, seedlings, and adult plants of varieties of wheat and barley inoculated with races of *Ustilago tritici* (Pers.) Rostr. and *U. nuda* (Jens.) Rostr., respectively, a rapid method of examining seed embryos and seedlings for loose-smut mycelium was developed. Seeds and excised portions of seedlings (embryos and seedling tissues) were macerated in a solution of potassium hydroxide, then boiled in a solution of cotton blue in lacto-phenol and mounted in a glycerin-water solution. Loose-smut mycelium, when present, was stained blue and could be recognized easily under the microscope in the transparent host tissue.

Seed of wheat which on examination was found to have uninfected embryos invariably produced adult plants that were free from loose smut. Conversely, seed of wheat having a high proportion of infected embryos might produce a corresponding number of smutted plants, a lower number, or none at all, depending upon the variety of wheat and the particular race of loose smut with which it was infected.

In instances where wheat seed having a high percentage of infected embryos produced few smutted plants there was evidence in one variety (Renown) of a continuous decline in infection throughout plant development. In other varieties the reduction in infection occurred only on germination of the seed. Consequently seedlings gave a more reliable index of adult-plant infection than did embryos.

Resistance to loose smut of wheat is determined by the particular race of loose smut used as inoculum, the reaction of the embryo, and the reaction of the tissues of the growing plant. A variety may be highly sus-

ceptible in the embryo stage to a race of loose smut and highly resistant in the seedling and adult-plant stages.

The prevalence of loose-smut infection in seed embryos and seedlings of resistant and susceptible varieties of barley was similar to that in adult plants.

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WOUND-PERIDERM FORMATION IN WHITE-PINE TREES RESISTANT TO BLISTER RUST[1]

B. ESTHER STRUCKMEYER AND A. J. RIKER

(Accepted for publication December 5, 1950)

White-pine trees selected by Riker et al. (7) for resistance to blister rust (*Cronartium ribicola* F.) have frequently recovered from stem infections. Hirt (6) and others have observed similar phenomena. This seemed important because it suggested (a) that the resistant trees were not escapes, (b) that recovery from infection was part of the evidence for resistance, (c) that during extreme conditions trees which became infected in side branches could inhibit the infection from reaching the main stem, and (d) that such trees even when infected in the main stem might develop cork layers behind the lesions. The histological picture of this recovery seemed worthy of study.

Reports of cork layers and their association with disease resistance appear in the literature. A few examples may serve as a background. Shaw (8) studied the resistance of apple and other rosaceous plants to fire blight. He observed frequently that a cork cambium developed and surrounded the entire stem. The new cork cells became suberized and served as barriers against further invasion of the bacteria into the tissue. Formation of these cork layers occurred sooner in the resistant than in the susceptible varieties. On leaves of the citrus, the histology of the lesions produced by *Sphaceloma fawcettii* Jenkins has been described by Cunningham (4). Within the spongy parenchyma of the leaf, cells formed from a cork cambium became suberized and prevented the hyphae from moving into the still healthy tissue. In certain resistant varieties of tobacco, cells of the root were capable of forming periderm when attacked by *Thielavia basicola* (Berk and Br.) Zopf. Conant (3) has shown that a cork cambium appeared which formed cork cells. They became suberized, separated the lesion from the normal tissue, and prevented invasion of the fungus into the healthy tissue. There was an optimum temperature at which periderm formation was most active. In the potato, Artschwager (2) described recovery from wounding and how it was affected by temperature and humidity. Wound periderm commonly develops from cells of the epidermis, cortex, or other parenchyma tissue capable of cell division (5).

The phloem tissue of white pine was described by Abbe and Crafts (1). Some of the phloem parenchyma cells were filled with tanninlike substances; others served as starch-bearing parenchyma.

The purpose of this paper is to describe the anatomy of the wound peri-

[1] Approved for publication by the director of the Wisconsin Agricultural Experiment Station.

This work was done as part of a cooperative project between the University of Wisconsin, the Wisconsin Conservation Department, and the U. S. Department of Agriculture. Mr. Herrling prepared the photomicrographs.

derm associated with the resistance to blister rust of certain selected white-pine trees.

MATERIALS AND METHODS

The tissues for this study were obtained from trees in the experimental blister-rust nursery (see Riker et al., 7) in central Wisconsin. Three-year-old wood was selected in the late fall of 1947 from susceptible and resistant trees, which had been artificially and severely inoculated 4 years earlier. The samples were 15 to 20 mm. long and were taken from lesions of both susceptible and resistant trees. They were fixed in formalin-aceto-alcohol, dehydrated with n-butanol, infiltrated, and embedded in paraffin. Sections 12 μ thick, cut on a sliding microtome, were stained with safranin and fast green.

OBSERVATIONS

Anatomy of the normal stem. The outer tissues of the stem were composed primarily of periderm. Adjacent to the periderm were the loosely arranged parenchyma, some of which had large nuclei and were filled with tannin (Fig. 1). Resin canals in different stages of development were scattered throughout the cortical region. Many of the sieve tubes adjacent to the cortex were being obliterated by the developing phloem-parenchyma cells. Such cells filled with tannin or starch were distributed among the differentiating sieve tubes which appeared in radial rows. The ray cells, usually uniseriate, were commonly granular and had prominent nuclei.

Anatomy of susceptible trees infected with blister rust. In material from the susceptible trees the fungus penetrated deeply into the tissue of the stem. Parenchyma and sieve tubes particularly were attacked by this organism and infection commonly progressed to the cambium (Fig. 2). Cells surrounding the resin canals were also invaded. The lesions showed masses of mycelia in sieve tubes, parenchyma, and ray cells. After severe invasion by the fungus, the parenchyma collapsed and, as the organism progressed deeper into the tissue, the phloem cells also collapsed into a mass of necrotic tissue and mycelium external to the cambium. Although a few of the tanniferous cells surrounding the lesion divided, there was no evidence of the formation of a cork cambium (Fig. 3).

Anatomy of resistant trees infected with blister rust. Trees called resistant were not immune from attack by the blister-rust fungus, since lesions were found occasionally, after extreme conditions, in the resistant trees. The first indication of infection in the stem was the presence of mycelium in the outer cortical cells of resistant stems (Fig. 6). In resistant trees the cork cambium was formed by the radial and tangential division of the cortical-parenchyma internal to the infected cells. The cork-cambial cells were rectangular and included about three tangential rows. The cork cells, differentiated from the cork cambium externally, were uniform in size and radially elongated, and the tangential walls soon became suberized. These

suberized layers of cork cells served as a barrier between the infected tissue and the healthy tissue.

The cork-parenchyma formed in radial rows on the inner sides of the cork cambium had large nuclei and were often granular. It appeared that

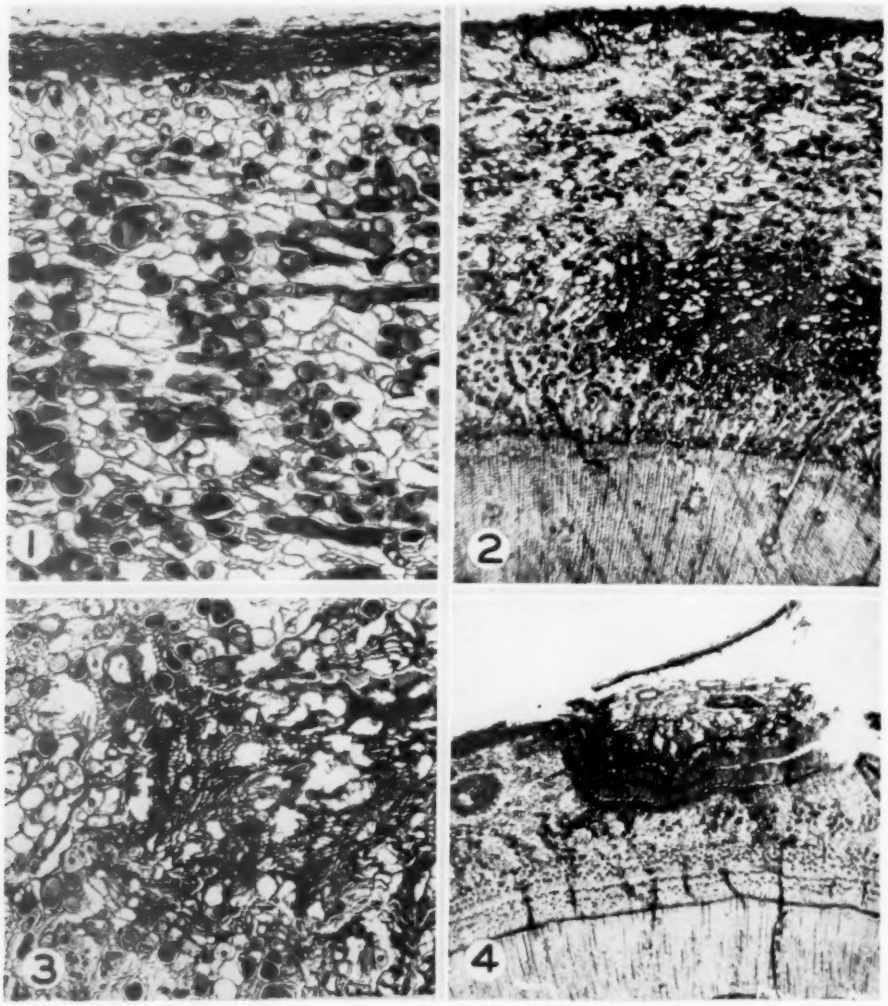

FIG. 1–4. Photomicrographs of cross sections of stems from white-pine trees. 1, normal trees; organization of cells of cortex and phloem. 2, tree susceptible to and infected by the blister-rust fungus; necrotic areas were induced by the fungus. 3, enlarged area of lesion in 2; a few cell divisions appear, but no evidence of differentiating cork. 4, tree resistant to the blister-rust organism; layers of cork cells and stone cells have differentiated from the cork cambium to form a barrier against further invasion into the tissue by the blister-rust organism.

some of the cork cells differentiated into stone cells so that there were alternating layers of suberized cork cells and stone cells, external to the cork cambium. The stone cells were isodiametric and arranged in five to seven

radial rows. The cell walls were heavily lignified, with conspicuous pits appearing as canals extending outward from the cavity of the stone cell to connect with the pits of the adjacent stone cell. These stone cells also served as protective layers against invasion of the fungus. Apparently cells formed from the cork cambium were able to differentiate as suberized cork cells or lignified stone cells (Fig. 5). Stone cells have been reported present in many kinds of bark (5).

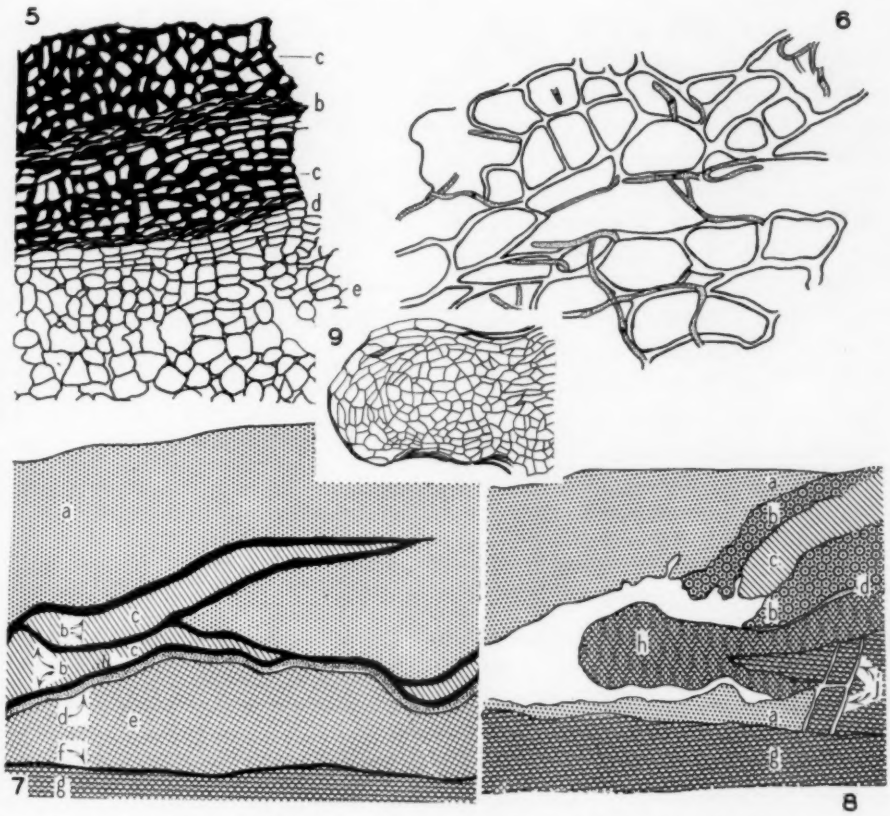

FIG. 5–9. Camera-lucida drawings of cross sections of stems in white pine (a, tissue infected or killed by blister rust; b, cork cells; c, stone cells; d, cork cambium; e, healthy tissue; f, cambium; g, xylem; h, callus tissue; j, rays extending from xylem). 5. Periderm under an infection, and normal tissue. 6. Hyphae in and between cortical cells. 7. Extension (b, c, d) of the periderm below diseased tissue after a tangential spread of the fungus beyond the original periderm. 8. Proliferation of callus tissue from cambium and ray cells extending over the xylem. When the fungus penetrated beyond the limits of the periderm, a callus mass (h) was formed to cover the xylem. 9. Cellular detail of callus tissue composed of parenchyma cells and differentiating cork cambium.

Infection did not develop severely in stems that formed a cork cambium from cortical cells, so that the bark periderm was continuous under the lesion. A barrier was formed before the mycelium had an opportunity to penetrate deeply into the tissue (Fig. 4). However, the cork cambium was not always formed from cortical cells. In trees with less resistance the

fungus penetrated beyond the cortex into the phloem cells, tanniferous cells, and ray cells. The phloem parenchyma cells and the tanniferous cells also were stimulated to form a cork cambium. Cork cells and stone cells were differentiated external to the cork cambium and cork parenchyma. Invaded cortical cells and resin cells outside the cork cells collapsed into a necrotic mass of cell walls and mycelium. No evidence appeared of mycelium among the cork parenchyma or any of the other cells internal to the cork cambium.

The cork cambium was stimulated only below the tissue that was infected with the fungus, so that occasionally the fungus progressed tangentially beyond the wound periderm, resulting in a new invasion into healthy tissue. However, a cork cambium soon differentiated below the newly infected region, separating it from the healthy tissue (Fig. 7). Resistant trees appeared to have the capacity to differentiate a cork cambium wherever an infection of the blister-rust fungus occurred, and thus to separate the invaded tissue from the healthy tissue.

In still less resistant trees the fungus penetrated deeply into the phloem tissue until it reached the cambium. This usually was apparent in stems in which the fungus had progressed beyond the limits of the wound periderm. When this occurred, ray cells (extending from the xylem) and the cambium proliferated to form a mass of callus. This grew over the damaged area as if starting to heal a wound (Fig. 8). Except for an occasional granular cell, most of the cells within the callus were meristematic (Fig. 9). These cells were protected from invasion of the fungus by the periderm that had formed on the outer surface of the callus.

DISCUSSION

White-pine trees have shown varying degrees of resistance to the blister-rust fungus (7). The present study has shown that when some of the resistant trees became infected they were able to set up a barrier against further invasion. The forming of a wound periderm in the stem beneath the infected tissue was a characteristic of the resistant trees which the susceptible ones lacked. In resistant trees in which infection occurred, a wound periderm was formed from the cortical tissue, but in stems with less resistance formation of wound periderm was slower and less effective. Stems of trees that were classed as susceptible showed no tendency toward wound periderm formation under the infections and eventually died. These anatomical studies showed that the degree of resistance of the trees to this disease was associated with their ability to induce the formation of a wound periderm under the infection.

The formation of wound periderm in resistant trees in relation to cause and effect is difficult to determine. Perhaps one can consider that following infection the fluctuating weather gives an advantage sometimes to the pathogen and at another time to the host. In comparison with many pathogens the blister-rust fungus grows slowly. Possibly the resistant trees first

reduce the activity of the fungus and then react to the injury by producing a wound periderm.

SUMMARY

White-pine trees resistant to blister rust were sometimes infected. In the stems of these trees a cork cambium developed that formed a wound periderm. This served as a barrier preventing the fungus from progressing further into the tissue.

The cork cambium was formed in resistant trees by tangential divisions of the cortical cells and phloem cells. External to the cork cambium, cork cells and stone cells were differentiated and cork parenchyma was formed internally.

After several layers of cork appeared, cells from the cork cambium were formed which differentiated into stone cells and became heavily lignified. Eventually the wound periderm contained alternating layers of suberized cork and lignified cells, with the cork cambium internal to these alternating layers.

The cork cambium was formed from cortical cells in trees with greater resistance to the fungus. In trees with less resistance, the fungus penetrated into the phloem parenchyma cells and ray cells before a cork cambium was formed.

In some still less resistant trees the fungus progressed to the cambium. When this occurred, cambial cells and ray cells extending from the xylem developed a callus which grew over the injured area. The callus was composed primarily of meristematic cells, with some cork cells on the surface. In susceptible trees, such wound periderm was not observed.

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A METHOD FOR THE COLLECTION OF MICROSCOPIC PARTICLES

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In the past, spores of fungi have been collected from their substrates by various means and with greater or less facility. For example, collection of uredospores of the cereal rusts has been tedious and wasteful of material, being accomplished by scraping the uredosori with a scalpel or by shaking the infected plants over paper and scraping together the deposited spores. The removal of spores from agar or liquid media has not been easy. McCallan and Wilcoxon[1] drew spores of *Neurospora sitophila* from nutrient agar cultures by a vacuum technique and trapped the spores from the air stream in water. Spore suspensions so obtained are unsatisfactory for many purposes. The replacement of the water trap by an apparatus able to collect spores from an air stream would be advantageous. Cyclone separators perform that task and function well for collecting spores and other microscopic plant parts.

A method has been developed to collect dry microscopic particles by using cyclone separators to recover spores from an air stream.[2] Several types of cyclone separators have been constructed, some of glass and some of metal, ranging in size from 4 cm. to 20 cm. in length. The larger models have been operated in conjunction with an electrically driven portable vacuum pump, delivering about 1.3 cfm. at 28.5 in. Hg. Sufficient air velocity is obtained for the smaller models from a rubber hand bulb or mouth suction. Several types of cyclone separators have been satisfactory.

1. Glass (Figs. 1, A, and 2, C). This consists of a hollow glass tube approximately 8 cm. long by 2 cm. in diameter. One end is sealed and rounded. The air intake, consisting of a tapered elliptical-shaped arm 6 cm. in length with a 2 mm. opening, enters tangentially at the other end of the body of the cyclone. The intake orifice has a serrated edge with four projections each about 1 mm. in length, constructed to prevent host tissue from either blocking the opening or becoming shredded by the air suction. Air is drawn by a vacuum pump, or by rubber hand bulb, from a tube 3 mm. in diameter, extending centrally into the cyclone separator to just below the point at which air enters the cyclone. This type is used for collection and immediate reinoculation of mildews, rusts, and other pathogens,[3] since the spores collected can be removed only by reversing the air flow and forcing the spores out of the cyclone in a dust cloud.

2. Detachable glass (Figs. 1, B, and 2, B). In basic construction this

[1] McCallan, S. E. A., and Frank Wilcoxon. The action of fungous spores on Bordeaux mixture. Contrib. Boyce Thompson Inst. 8: 151–165. 1936.

[2] Tervet, Ian W. A technique for the collection of dry spores from infected plants. (Abstr.) Phytopath. 40: 874. 1950.

[3] Ian W. Tervet and R. C. Cassell. A simple method for collecting and inoculating rusts in race identification studies. (Abstr.) Phytopath. 40: 874. 1950.

model is similar to No. 1, differing from it in having the body of the separator in two parts joined by a tapered ground-glass sleeve. The particles are collected in the lower part of the separator where they can be stored or readily removed to other containers.

3. Large aluminum (Figs. 1, C, and 2, A). This model operates on the principle of the preceding types. Its metal construction prevents breakage, and its larger size allows use where more extensive spore-bearing surfaces are available. The cyclone is machined from aluminum and has an overall length, including the collecting vial, of 20 cm. The separation of the spores occurs in a conical chamber 2 cm. at its greatest diameter where

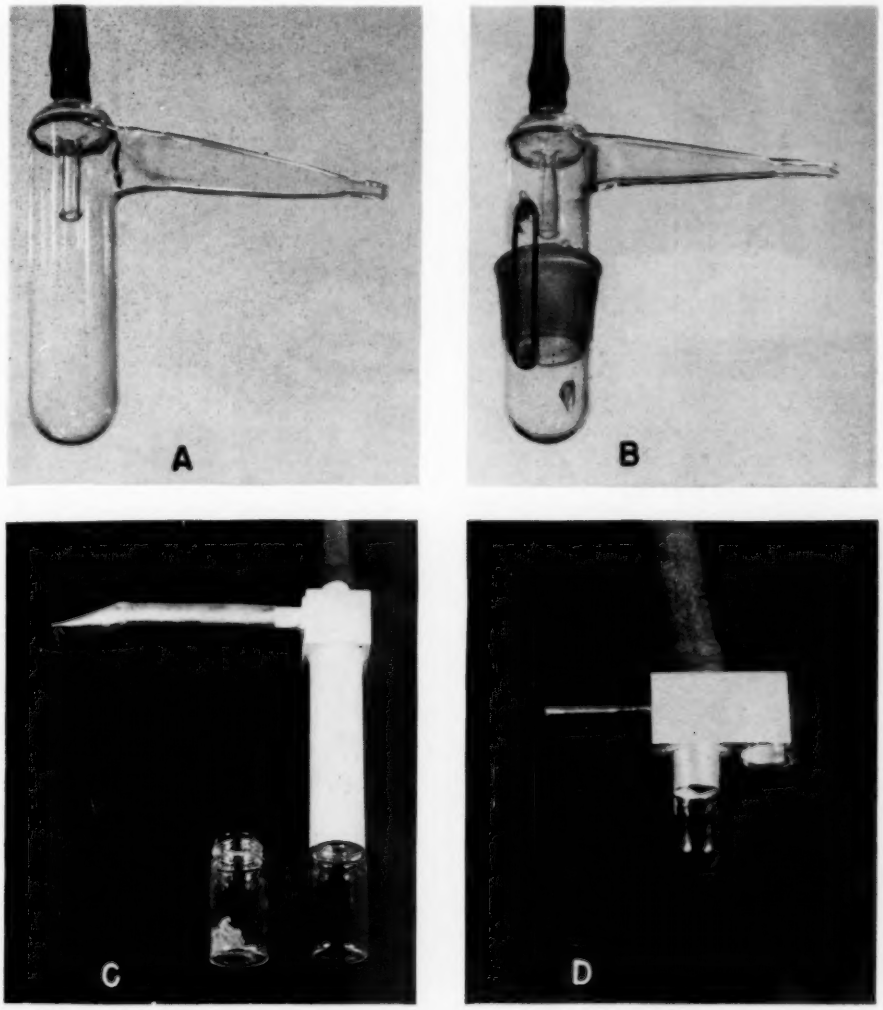

FIG. 1. Types of cyclone separators: A, glass cyclone; B, detachable glass cyclone, showing rubber band retainer; C, large aluminum cyclone with spores in collecting vial; D, small aluminum cyclone.

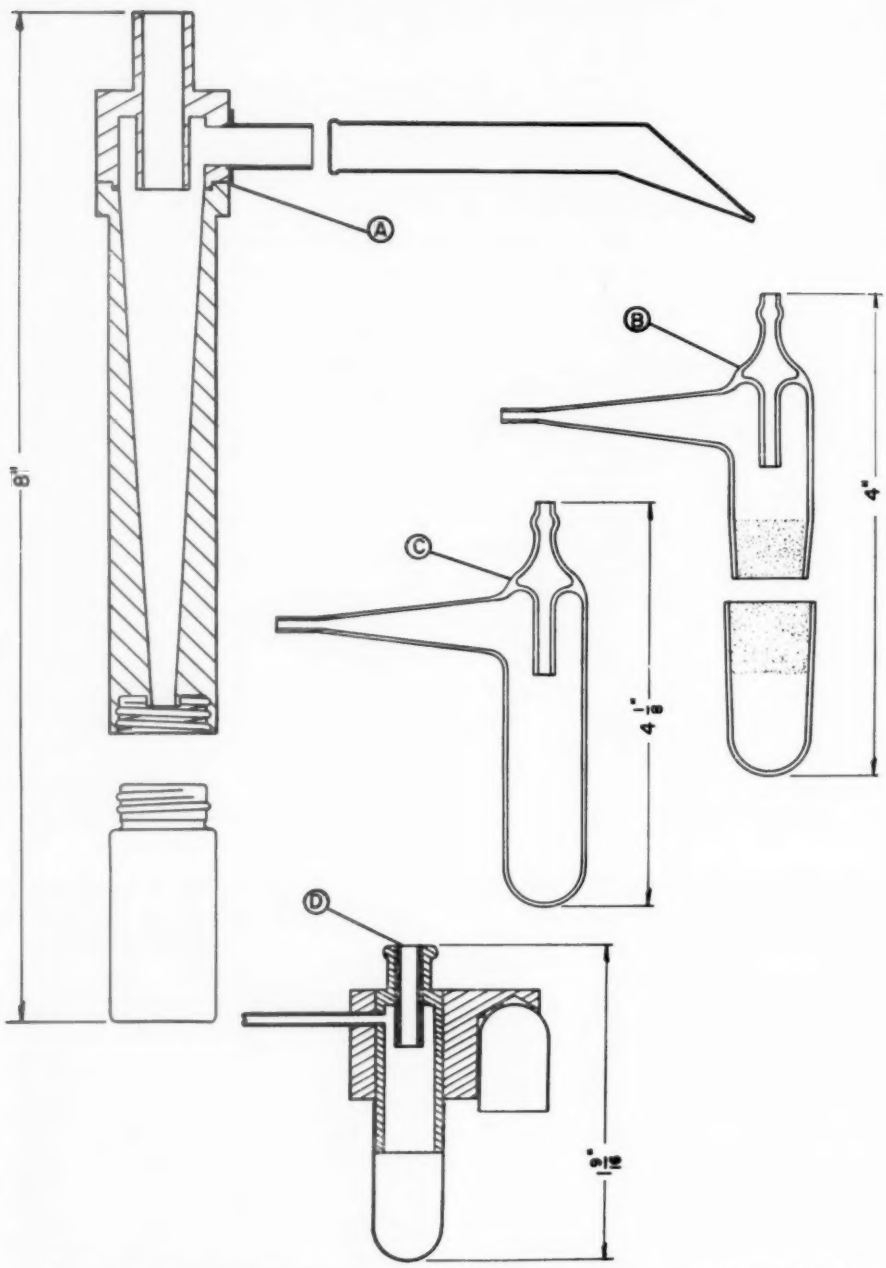

FIG. 2. Schematic drawing of cyclones: (A) Large aluminum, (B) detachable glass, (C) glass, (D) small aluminum.

the spore-laden air enters, and 8 mm. in diameter where the spores settle out into the collecting vial. The cone, 11.5 cm. long, has a highly polished interior so that spores fall freely into the glass vial. The pickup is of $\frac{3}{8}$ in.

copper tubing to which has been soldered a brass fantail having an opening of approximately 2.5 cm. × 1 mm. This model operates in conjunction with a portable vacuum pump with a capacity of 1.3 cfm. at 28.5 in. Hg.

4. Small aluminum (Figs. 1, D, and 2, D). This model was developed for field use and is operated either with a rubber hand bulb or by mouth suction. The body is aluminum and resembles the detachable glass model on a reduced scale. The lower part of the cyclone where the spores are collected consists of the longer portion of an ordinary No. 00 gelatine capsule. Incorporated into the assembly is a channel for holding the top, corresponding to the capsule being used in the collection.

Use of cyclone separators. By connecting a cyclone separator to a vacuum pump and placing the intake opening of the cyclone close to a spore-bearing substrate, spores are sucked off into the air stream and deposited in the separator. Spores can thus be readily collected from infected plants, provided the spores are not sticky and are adapted for wind dissemination. When a glass extension tube is attached to the intake of the cyclone, dry spores of many fungi growing on artificial substrates can be collected. A further limitation of the cyclone separator in use is that collection of the spores from the air stream is not efficient when the diameter of the spores is less than about 4 μ. When larger spores are collected these devices are highly efficient. Spores as different in size as *Aspergillus tamarii*, 5–6 μ in diameter, and *Helminthosporium* spp., circa 100 μ in length, have been collected from nutrient culture. Results from several experiments under controlled conditions of air pressure and air flow, using uredospores of the cereal rusts, indicate that recoveries from all the cyclones described approach 100 per cent. In three such experiments with the glass cyclone, spore recovery averaged 98.6 per cent of the starting sample of 500 mg., irrespective of the pressure employed within limits regarded as optimum. Above a pressure drop of 31 in. of water and below 2.5 in. of water, losses ran in excess of 1.4 per cent. It appeared that some uredospores were lost at the exhaust at the greater pressure drop, whereas efficiency of the pickup dropped at the lower limit of 2.5 in. of water.

SUMMARY

The use of cyclone separators makes possible the collection of microscopic plant parts in a relatively pure form and in a highly efficient manner.

Mildew conidia, uredospores of the cereal rusts, or spores of many other plant pathogenic fungi fructifying on infected plant tissue can be collected without the use of a suspending medium. Similarly, pollen grains, or in fact any microscopic particles larger than 4 μ, can be collected.

The technique can be used for spores produced on pure cultures on artificial substrates, thus allowing the collection of dry spores of microorganisms grown in culture tubes, Petri dishes, or other containers.

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THE USE OF CYCLONE SEPARATORS IN RACE IDENTIFICATION OF CEREAL RUSTS

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Recognition of the occurrence and distribution of physiologic races of cereal rusts is of great importance in the development of control measures for these diseases, particularly when control is sought by the use of disease-resistant varieties. The method used in the identification of races is essentially the same for all the rusts, differing only in the selection of the differential host varieties required for each rust species or variety. Usually field collections of rust are first propagated on a susceptible variety and then transferred to the differential series of varieties for identification of the race.[1]

The field sample ordinarily consists of several pustules. For the initial transfer to a susceptible variety, the spores are scraped from the pustules with a scalpel; the scalpel, with its spore deposit, is then rubbed over the moistened leaves of the host plant. Propagation from the primary infections that develop after 10 to 14 days is made by brushing the infected leaves across the moistened leaves of the differential varieties. In practice, identification of a field collection requires from 20 to 28 days from the time the sample has been received at the laboratory for analysis.

The field collections usually consist of a few leaves or stems bearing from one to several pustules. A substantial loss in spore viability sometimes results during transit from field to laboratory. That transit period may be several days, during which the spores are often exposed to high humidities and high temperatures. Transfer of the spores by scalpel usually involves the mixing of spores from more than one pustule. When more than one race occurs in a sample, a mixture of races will develop on the differentials, requiring additional effort and time for their separation.

The development of small cyclone separators,[2] and their dual use both as collectors and disseminators of rust spores,[3] has facilitated the handling of uredospores and thus made the task of rust race identification easier. This paper describes some methods that have proved successful in studies with cereal rusts.

The methods outlined below have reference primarily to stem rust of wheat, but other cereal rusts and many other plant pathogens can be handled in a similar manner.

[1] Stakman, E. C., M. N. Levine, and W. Q. Loegering. Identification of physiologic races of *Puccinia graminis tritici*. U. S. Dept. Agr., Bur. Ent. and P. Q. E-617. 1944.

[2] Tervet, Ian W. A technique for the collection of dry spores from infected plants. (Abstr.) Phytopath. 40: 874. 1950.

[3] Tervet, Ian W., and R. C. Cassell. A simple method for collecting and inoculating rusts in race identification studies. (Abstr.) Phytopath. 40: 874. 1950.

CYCLONE SEPARATORS AS SPORE COLLECTORS AND DISSEMINATORS

Spores can be collected from field samples of wheat stem rust and immediately dusted on the differential varieties, so that the primary increase

FIG. 1. Glass cyclone spore collector connected to a suction pump.

of inoculum on Little Club wheat is omitted. For this purpose a glass cyclone 8 cm. long and 2 cm. in diameter is used (Fig. 1). About 0.5 gram

FIG. 2. Cyclone collector removing rust spores from a field sample. Collector partially filled with talc before removal of spores.

of talc is first collected in the cyclone, then the uredospores from the field sample are sucked into the cyclone and become mixed with the talc (Fig. 2). Additional talc (from 0.5 to 1.0 gm.) is added and the spore-talc material is thoroughly mixed. With this type of glass cyclone, reversal of the air flow permits the use of the apparatus as a duster, so that the spore-talc mixture can be distributed upon the plants of the differential varieties in an incubator (Fig. 3). Seven pounds air pressure is sufficient for dusting;

FIG. 3. Cyclone collector used as a duster for inoculation by reversing the air flow. (Note small glass dust spreader attached to the nozzle of the cyclone.)

minor adjustments in the rate of dusting can be made by manual constrictions of the rubber inlet tube. The tube can be operated in such a manner as to produce a puffing effect of the air through the cyclone duster and thus completely free it of spores. The differential varieties are placed in an incubator prior to dusting, and following inoculation the cover is quickly replaced on the incubator. The chance of contamination of the differentials is slight, for the number of spores used in the spore-talc mixture is very much less than the number occurring on the infected Little Club plants normally used for inoculation of the differentials.

The efficiency of collection and distribution of uredospores is well illustrated by the following summary of a series of tests. Spores were collected from single susceptible-type pustules on greenhouse-grown wheat plants and after being mixed with talc were dusted on sets of the wheat stem rust differentials. Spores were obtained from 28 type 3– to 4 pustules and were

used to inoculate 28 sets of differentials totalling 4704 plants. The inoculated plants were incubated in a standard rust incubator at 65° F. for 12 hr. An average of 1.3 pustules per leaf developed, which is an infection rate sufficiently heavy for race identification. Single resistant-type pustules can be isolated with ease from the differential plants if race mixtures are observed. This eliminates the necessity of rebuilding the quantity of separated spore materials on Little Club for further inoculation work. Where pustules of infection types 1 or 2 appear on the same leaf with those of types 3 or 4 of another race, the cyclone separator may be used to isolate the spores from enough resistant-type pustules to permit immediate reinoculation of a set of differentials. In limited tests in which uredospores from resistant-type pustules were used to reinoculate six differential varieties known as the "half-series," the following results were obtained. Single pustule isolations of type 2– pustules reproduced about one pustule for every four seedling leaves inoculated. Single type 2++ pustules have reproduced at a rate as high as 1.2 pustules per leaf. Spores from nine type 1 pustules used together as the source of inoculum have reproduced at a rate of 2.1 pustules per leaf.

A detachable glass cyclone separator of the same size as the one described above has been developed.[4] A sufficient quantity of spores can be collected from a set of differentials, or a "half-series," for storage purposes. After the rust spores have been collected in the base of the detachable cyclone they may be transferred to small glass vials which can be tightly sealed. Uredospores collected for delayed inoculation may be temporarily stored by detaching the base of the cyclone and inserting a rubber seal (Fig. 4). The base can be replaced on the upper portion of the cyclone

FIG. 4. Detachable glass cyclone collector. When the base is removed and sealed it may be used as a spore storage unit.

when inoculation is completed, with no further handling or loss of spores.

In order to evaluate the practicability of having field samples contain only spores from a single pustule, or spores from two or three small pustules, a small aluminum cyclone separator operated by lung suction has been

[4] Tervet, Ian W., A. J. Rawson, E. Cherry, and R. B. Saxon. A method for the collection of microscopic particles. Phytopath. (In press.)

built (see footnote 4). The spores separated by this cyclone are collected in the base portion of a No. 00 gelatin capsule in which a small quantity of talc (about 40 mg.) has been placed. After the spores are collected, the capsule is removed, sealed, labelled, and sent to the identification laboratory. Limited tests with the apparatus have been promising, but additional tests in the field under a diversity of environmental conditions are necessary before recommendations can be made. Some decided advantages lie in the use of the small cyclone separator for single pustule field collections: 1) The spores can be collected when dry; 2) they are stored in talc which tends to stabilize the moisture content of the spores; 3) they are ready without further treatment for inoculation of the differentials; and 4) the chances of race admixtures are greatly minimized.

CONCLUSIONS

Race identification of cereal rusts can be facilitated by the adoption of cyclone separators for the collection of rust spores and their transfer to the host differentials. The period required for identification has been reduced to 10 to 14 days by eliminating the necessity of a primary increase of the rust on a susceptible variety. Race mixtures and further purification of a single rust collection can be almost eliminated by utilizing small-quantity field samples with the cyclone separator. The separator is advantageous in making the process of collecting spores and inoculating plants time-saving and less laborious. It makes efficient use of tiny quantities of spores by evenly distributing them with talc under light pressure upon the plants.

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ANTHER SMUT OF CARNATION[1]

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Ustilago violacea (Pers.) Fuckel has long been recognized as a pathogen of the Caryophyllaceae. Since the classic works of Tulasne, Cornu, Giard, and Magnin, development of rudimentary stamens in female flowers of *Melandrium album* (Miller) Gke. and other species of the pink family often has been attributed to the activities of this anther smut. Observing that the normal pollen was replaced by smut chlamydospores, Magnin and Giard (12) aptly designated the phenomenon as "castration parasitaire." Little is known, however, concerning the ravages of *U. violacea* on varieties of the perpetual-flowering carnation, *Dianthus caryophyllus*.

In 1936 anther smut of carnation, previously believed to be unimportant, necessitated the destruction of 70 per cent of the blooms prepared for marketing by an English nursery and subsequently provoked the first in a series of investigations on early symptoms and control of the disease (19). Three years prior to this serious outbreak, Conners (4) reported the smut as attacking Canadian carnations.

Ustilago violacea was first noted by H. E. White as occurring on carnations in the United States in July, 1948 (16). The original station in eastern Massachusetts is to date the only authenticated location of carnation anther smut in this country.

Mycological studies of *Ustilago* species pertinent to this paper were undertaken by Harper (8), Kniep (11), Wang (17), Bauch (2), Werth and Ludwigs (18), Magnin and Giard (12), Erlenmeyer and Geiger-Huber (6), and others.

SYMPTOMS AND SIGNS OF DISEASE

Infected carnation plants are weakened and stunted. Axillary developopment increases, internodes are short, leaves are small, and buds are squat. Flowers are numerous but small and the calyx tube tends to split. Staminodia often revert; they vary from attractive petaloid devices, so noticeable in normal carnations, to stamens with normal anthers. Smutted flowers appear sooty because purplish-black chlamydospores replace the pollen in the anther sacs. The gynoecia, or carpels, are entirely suppressed by the fungus.

Symptoms are slow in appearing and their detection in the early stages of disease is difficult. In fact, macroscopic examinations of smutted carnation plants demonstrate few abnormalities prior to the appearance of smutted blooms.

[1] Contribution No. 751, Massachusetts Agricultural Experiment Station.

INOCULATION STUDIES

Since *Ustilago violacea* has been shown by several investigators (7, 21) to have physiologic specialization, the source of inoculum for natural infection is generally conceded to be smutted flowers of the same species in the locality.

White (19) noted that infection of carnations by anther smut was favored by retention of stagnant moisture in leaf axils and by the damp atmosphere of beds in glasshouses. In a later paper by White (20) it was reported that infection was not obtained through unbroken surfaces, the wounded or cut surface of an old plant, or through roots. True flower infection is most frequent in nature, followed in importance by seedling and axillary bud infection (1). Baker, in the same article (1), also questioned the theory of seed transmission held by many earlier investigators, since ovules always abort when ovaries are invaded. *Melandrium album* develops no normal fruits when parasitized by *U. violacea* (14).

One of the more important avenues of infection detected by earlier workers appears to be cut ends of stems left when carnations are harvested or in the "stopping" of first-year plants (20).

In natural inoculations observed by the writers, spore production usually commenced with the opening of the first flower of a diseased plant and continued in blossoms appearing later. Chlamydospores may be disseminated from a single bloom for as long as 3 weeks. Subsequent normal blooms were rare on carnation plants that had once borne smutted flowers. The greatest number of spores which came to rest on plants were found on leaves and in their axils, and on flowers and flower buds. Most significant are the masses of spores often noted in opposing acaulescent leaf axils left by the common practice of breaking off inflorescent axes at nodes. Dissemination of spores is enhanced when smutted flowers are disturbed.

TABLE 1.—*Susceptibility of various parts of Dianthus caryophyllus to infection by anther smut*

Mode of inoculation, and material inoculated	Number inoculated	Percentage infected
Suspension of chlamydospores		
Bud (open calyx tube)	60	87
Flower	36	92
Excised stem (new growth)	49	86
Leaf, leaf axil, and stem		
Rooted cutting; exposed tissue	50	70
Do nonexposed tissue	35	3[a]
Adult plants; exposed tissue	44	19
Do nonexposed tissue	40	0
Infested soil; rooted cuttings	49	8
Infested sand		
New cuttings, Hormodin treated	69	3
Do not treated	49	14

[a] A single plant became infected; examination revealed removal of cuticle in area of infection.

Spore counts on ventral surfaces and axils of 38 leaves selected at random from a plot of smutted carnations yielded an average of 17 chlamydospores, prior to overhead watering, and an average of 41 spores per leaf and leaf axil after a 2-min. watering of average intensity.

Data regarding methods and results of artificial inoculations are summarized in table 1.

Floral susceptibility. Both buds and flowers of carnation were employed for floral inoculations. Buds in which the calyx tube had been open for less than a day were inoculated by means of a spore suspension[2] placed on exposed petal extremities. Similar suspensions were placed also in centers of expanded flowers. Of the 60 buds inoculated, 87 per cent became smutted, and 92 per cent of the 36 flowers treated succumbed to the disease.

Susceptibility of vegetative parts. Artificial inoculations of vegetative portions were performed in duplicate both on recently rooted cuttings and on established plants. In one series spores were placed on unbroken surfaces, in the other series inoculum was introduced to wound tissue. In each series, stems, leaves, and leaf axils were inoculated and spore suspensions held in place by means of absorbent cotton. Excised ends of stems were also inoculated but no retainers were necessary.

Rooted cuttings proved three to four times more susceptible to anther smut than did established plants. Seventy per cent of 50 cuttings inoculated on exposed tissue became infected, but only 19 per cent of 44 established plants inoculated in a similar manner ultimately had symptoms of disease. Only a single plant in this series became infected through an unbroken surface. Microscopic examination of the infection area of this cutting revealed that some of the cuticle had been damaged by red spider. Apparently, inoculation is possible after removal of the protective cuticle.

Forty-three of the 49 excised stems, obtained by breaking off five or six sections (internodes) from new growth, were successfully inoculated through their newly exposed ends.

Infestation of soil. Chlamydospore-infested soil and sand were tested as possible sources of infection. Following cultural practices, where feasible, new cuttings were placed 2 in. apart in moist sand sown with approximately 10,000 spores per sq. ft. Relative humidity above the plots varied from 92 to 98 per cent, and air temperature varied from 12° to 20° C. As a control, other cuttings were set in sterile sand under similar conditions of humidity and temperature. Half of each group was treated with Hormodin No. 1.

Rooted cuttings were set in a sandy loam dusted with the same concentration of spores as had the sand. A control series of rooted cuttings was also set in untreated loam.

[2] In all cases, except the soil series, spore suspensions were consistent and were composed of approximately 500–700 chlamydospores (less than 30 days old) suspended in 0.2 ml. of glass-distilled water.

One-seventh, or 14 per cent, of the untreated cuttings rooted in infested sand became smutted, whereas only 3 per cent of 69 cuttings treated with Hormodin No. 1 were attacked. Eight per cent of the 49 established cuttings set in contaminated soil ultimately produced smutted blooms.

Cuttings taken from carnation plants known to be smutted did not produce roots so readily as did those from healthy plants. Attempts to destroy the pathogen within living suscept tissues by hot-water immersion of cuttings failed; the host was invariably killed before the fungus was destroyed.

Smut inoculum from other host species. In an attempt to induce infection by other strains of *Ustilago violacea*, inoculations of both flowers and excised stems were made with chlamydospores obtained from *Arenaria serpyllifolia* L. and *Silene antirrhina* L. Though 20 per cent of the spores proved viable and readily formed germ tubes on carnation, no infection was produced.

INCUBATION AND GERMINATION STUDIES

If conditions are favorable the active stage of germination may begin shortly after formation of chlamydospores and before their dissemination. The incubation period apparently varies with the temperature and moisture present. A majority of spores on exposed tissue, in water held by leaf axils, or in centers of flowers, gave rise to promycelia within 24 hr. Spores on uninjured epiderms, free from standing water, germinated well in 4 to 6 days. Many spores which failed to leave smutted blooms were observed to produce promycelia *in situ*.

All spore lots employed in the following investigations were between 3 and 5 weeks old at the start of the experiments.

Temperature factor. Cardinal temperatures for germination were determined by germinating spores in hollow-ground slides at a series of temperatures, 1° C. apart, ranging from 3° to 39° C., in glass distilled water. Five trials were performed on different dates and the percentage of germination for each trial was raised to bring the maximum percentage for any trial equal to the index number 100. Not less than 500 spores were counted for each degree of temperature. All observations were made after 48 hr., and upon the completion of the five series, index numbers were averaged. As in all instances in this paper, germination was arbitrarily defined as development of promycelia. Cardinal temperatures were found to be as follows: minimum 5° C.; maximum, 36° C.; and optimum, between 16° and 22° C. These temperatures compare favorably with those reported by other workers for *Ustilago* species. Davis (5) found the optimum temperature for germination of *U. striaeformis* spores to be 22° C.; the minimum and maximum, 7° and 35° C., respectively. Jones (9) noted that spores of *U. zeae* germinated best at temperatures ranging from 26° to 34° C., while the chlamydospores failed to produce promycelia below 8° or above 38° C. In another article (10) the same investigator reported the following cardinal temperatures for spores of *U. avenae:* minimum, 4° C.;

optimum, between 15° and 28° C.; and maximum, 34° C. It should be pointed out, however, that Jones arbitrarily defined optimum temperature for germination as that range over which sporidial production takes place in 24 hr.

Certain trends were observed for the germination of air-dried spores stored in glass vials at 20°, 0°, and −25° C., for periods increasing from 24 hr. to 2 months. At all three temperatures percentage of germination during the first week was relatively high. After this initial period, percentage of germination fell but remained steady for about 1 month, then gradually declined. In the final observations, spores had germinated after 2 months in the following percentages: 20° C., 9 per cent; 0° C., 14 per cent; and −25° C., 4 per cent. Smutted flowers kept under the same conditions yielded spores germinating in percentages parallel to but approximately 5 per cent higher than those of the above series. In all cases, germination was tested by the procedure stated under cardinal temperatures.

Moisture factor. Employing a method adapted from Clayton (3) for accurately controlling high relative humidities, the writers observed 2 per cent germination at 94 per cent R.H., increasing to 41 per cent at 99 per cent and 100 per cent R.H., but only 0.2 per cent germination at 91 per cent R.H. and none below (Table 2). Spores were germinated on dry glass

TABLE 2.—*Humidity and soil-moisture relations affecting germination of chlamydospores of Ustilago violacea*

Relative humidity	Average[a] germination	Water-holding capacity of soil	Average[a] germination
Per cent	*Per cent*	*Per cent*	*Per cent*
90	.....	20	1
91	0.2	30	15
92	0.4	40	23
93	0.4	50	32
94	2.0	60	26
95	5.0	70	24
96	10.0	80	18
97	16.0	90	2
98	25.0		
99	41.0		
100	41.0		
H_2O	25.0		

[a] Average for 5 trials.

slides at 20° C. for 48 hr.; a companion series of spores germinated in water for the same period of time yielded a figure of 25 per cent. Clayton (3) noted that chlamydospores of *Ustilago hordei* and *U. nuda* could germinate at relative humidities of 95 per cent and 100 per cent, but not at or below 93 per cent.

The relation of soil moisture to chlamydospore germination was studied by means of a procedure devised by Jones (10). Spores were placed on layers of agar between filter papers in soil samples removed from carnation beds and containing different amounts of moisture. Five determinations

for wilting percentage on the same soils yielded a mean of 10.5 per cent. Germination was found to be highest at 50 per cent of the soil water-retaining capacity, diminishing gradually between 60 per cent and 80 per cent, and declining markedly at 90 per cent (Table 2).

Microscopic examinations revealed that chlamydospores germinating in water formed uninucleate sporidia freely on their promycelia, the sporidia, promycelial cells, or a sporidium and cell of the promycelium copulating with or without formation of copulation tubes. As reported by other workers (2, 8, 11) there appeared to be a sexual differentiation since sporidia arising from the same cell would not fuse, nor would they unite with their parent cells.

Though the paired bodies increased somewhat in volume, there was no apparent cytoplasmic interchange and the nuclei remained in their respective cells. Copulating units rarely were noted as forming germination tubes and then only in the presence of wounded suscept tissue.

Chlamydospores germinating in a highly moist atmosphere produced very few sporidia, the promycelial cells forming germination tubes directly.

INFECTION OF DIANTHUS CARYOPHYLLUS

Penetration of suscept tissues by the fungus is interpreted to be the commencement of infection. The infection hyphae were uninucleate at first but seemed to become multinucleate soon after incursion of host. In the floral regions, the hyphae which segment, round up, and form chlamydospores were binucleate.

Germ tubes readily penetrated all aerial parts of carnation plants through wounded epiderms. Uninjured vegetative tissues were not invaded, nor were intrusions through stomata noted. However, several plants were successfully inoculated through unimpaired surface tissues where the cuticle had been removed by careful scraping. The flower seemed to be most susceptible of all plant organs inoculated. Infection was easily effected from the moment the calyx tube opened to expose petals and staminodia.

In recently rooted cuttings known to be infected with smut, mycelium was most abundant in nodal regions and in leaf bases. Apparently the fungus keeps pace with the developing plant since hyphae were detected in tissues immediately beneath apical meristems. Mycelium in plants bearing smutted flowers occupied the same regions as noted in cuttings and did not seem to favor any particular tissue. Hyphae were seen in the pith and cortical tissues as well as in and surrounding vascular elements. The fungus is intercellular; no true haustoria were seen. In internodes mycelium is sparse and hyphae are long and unbranched; in nodes, it is abundant and hyphae are short, convoluted, and branched. Smut mycelium is most abundant in the torus and androecium of carnation blooms. There appears to be some degeneration and disappearance of mycelium from older and lower portions of the plant.

Monosporidial infections were localized and did not result in the formation of chlamydospores. It can, therefore, be assumed that infection of carnation by *Ustilago violacea* is accomplished by haploid, sexually differentiated hyphae, and that fortuitous meeting and subsequent union of hyphae of opposite sex give rise to the systemic mycelium which bears chlamydospores.

GENERAL DISCUSSION AND CONCLUSIONS

The details of the life history of *Ustilago violacea* (Pers.) Fuckel on *Dianthus caryophyllus* are essentially similar to those of the same species in its relationship to other hosts. Certain observations and results should be emphasized which may or may not be divergent from the conclusions reached by other investigators.

The pathogen attacks its host, carnation, only through injured surfaces and flowers. In time the fungus becomes systemic and sori are formed in the anthers, where pollen is replaced by a sooty mass of chlamydospores. The hyphae which segment into spores are binucleate, whereas the vegetative mycelium is generally multinucleate. Though Werth and Ludwigs (18) could find no binucleate mycelial cells, the present observations agree with those of Wang (17) for *U. violacea* on *Dianthus morspessulanus*.

Only observations were made of the intercellular nature of the smut fungus. In parasitized blossoms the stimulation of stamen development from primordia present is thought by some workers to be a result of hormone action (6, 15). The effect is best seen in dioecious species of the Caryophyllaceae.

Chlamydospores germinating in water produced sporidia freely, but a relative humidity above 91 per cent favored direct formation of germ tubes and, apparently, infection, more than water contained in leaf axils and other portions of carnation plants. Present studies showed the percentage of germination to be markedly reduced by germinating spores in water as against in a very moist atmosphere. Atmospheric moisture immediately adjacent to transpiring tissues often approaches saturation (13, p. 195). Germination, which is actually retarded in saturated soils, is highest when the moisture-retaining capacity of soil is about 50 per cent.

Chlamydospores evidently are capable of overwintering outdoors in the most extreme northern latitudes of the United States. Four per cent of chlamydospores stored at $-25°$ C. for more than 2 months germinated. However, control of anther smut should offer few problems since the pathogen does not attack an uninjured plant except through the flower and there are strong suggestions of physiological specialization. Also, optimum temperatures for germination of spores average 5° to 7° C. higher than those normally employed in the culture of carnations.

Though the early stages of carnation smut are difficult to detect macroscopically, the squat buds and split calyx tubes afford a ready means of recognition before smut spores are discernible. Close inspection of plants

during the warmer months of the year is advised. Diseased plants should be removed at once and burned; soil treatment for smut should be necessary only in cases of severe infestation.

SUMMARY

The life cycle and action of anther smut, *Ustilago violacea* (Pers.) Fuckel, in parasitizing carnation, *Dianthus caryophyllus*, examined for the first time, is essentially similar to the reported effects of this fungus on other members of the pink family. Carnation plants infected with smut bear flowers containing no carpels but an abnormal quantity of anthers in which pollen has been replaced by chlamydospores.

Cardinal temperatures for germination of anther smut spores are as follows: minimum 5° C., maximum 36° C., and optimum between 16° and 22° C.

Air-dried spores germinated readily after being subjected to temperatures of 20°, 0°, and −25° C. for as long as 2 months. Smutted flowers stored at the same temperatures yielded spores which germinated in slightly higher percentages than the air-dried chlamydospores.

One per cent of the spores germinated at a relative humidity of 94 per cent and germination increased to 41 per cent at 99 per cent R.H. Twenty-five per cent of the spores of a companion series germinated in water. A difference is seen in behavior between spores germinated in a very moist atmosphere and those germinated in water. In high relative humidity, infection tubes were normally produced directly from the promycelium, while water favored the formation of sporidia.

Spores germinated in highest percentages when the water-retaining capacity of soil was 50 per cent. A gradual decrease in the number of spores germinating was noted between 60 per cent and 80 per cent of the soil water-holding capacity, and at 90 per cent, germination declined abruptly.

Except for flowers, infection cannot be induced through unbroken surfaces. Flowers, buds, and ends of excised stems, in that order, are the most susceptible areas. Young growth is much more liable to infection than is old growth. Infested sand and soil may be a source of inoculum.

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PHYTOPATHOLOGICAL NOTES

Helminthosporium victoriae as a Leaf-Spotting Pathogen on Oats in Louisiana. J. G. Atkins, Jr. Although leaf spots were listed as a symptom of Helminthosporium or Victoria blight of oats in Louisiana in a previous publication,[1] they have not been reported by other workers in the southern States. This leaf phase of the disease seems to be rather important in Louisiana.

In April 1948 an unusual leaf spot was observed on such varieties as Quiney Red, DeSoto, Victorgrain, Traveler, and Letoria in a nursery test at Baton Rouge, Louisiana. Since the spots were found only on varieties of Victoria parentage known to be susceptible to Helminthosporium blight, and not on the Bond or Red Rustproof varieties, *Helminthosporium victoriae* was considered as a probable pathogen. In a laboratory examination of the leaf spots, conidia of a Helminthosporium were present. Conidia of *Alternaria* and *Epicoccum* were also frequent.

The leaf spots vary greatly in size but are often as large as 0.5–1 × 1–3 cm. They are oval to elongate with somewhat indefinite or irregular margins, greenish-gray to brown, often appearing as eye spots. Extensive necrosis results when the spots are numerous and coalesce. When the leaf spotting involves the midrib, the leaves bend downward in characteristic fashion, particularly when the lower third of the leaf blade is infected. Leaf spots tend to be larger and more characteristic on varieties with broad leaves, such as DeSoto.

Conidia are not numerous on necrotic areas. Freshly collected spotted leaves held in moist chambers have large numbers of conidia, which differ considerably from the typical conidia of *H. victoriae* taken from blackened nodes or from abundantly sporulating cultures; they have lighter or faded coloration, slighter curvature, and more uniform length, width, and septation. Conidia from leaf spots and from nodes average, respectively, 14.2 × 65.8 μ and 14.6 × 62.7 μ.

In 1948 and 1949 *H. victoriae* was rather consistently isolated from a large number of leaf spots. *H. avenae* and a few other fungi were sometimes encountered. When these isolates of *H. victoriae* were compared with those from darkened nodes, only minor cultural differences were apparent, and isolates from leaf spots were as pathogenic on Victorgrain seedlings as those from nodes. Soil was artificially infested with an isolate prior to planting in the greenhouse tests. The percentage of seedlings with foliar striping was used as a measure of pathogenicity. Larger Victorgrain oat plants growing in the greenhouse were inoculated by atomizing with a dilute conidial suspension of *H. victoriae* isolated from leaf spots and nodes, then held in a moist chamber for 48 hr. Leaf spots similar to those observed in the field were evident a few days after inoculation (Fig. 1).

[1] Stamper, E. R., John G. Atkins, Jr., and John P. Gray. Helminthosporium blight of oats in Louisiana. La. Agr. Exp. Sta. Bul. 431. 1948.

In Louisiana, particularly in the Baton Rouge area, leaf spots generally appear about the time of head emergence and become severe by the time the dough stage is reached. Nearly 100 per cent of the leaf area may be invaded and killed by *H. victoriae,* alone or in combination with crown rust. It should be pointed out that although oat plants with the Victoria type of reaction to crown rust are resistant to crown rust, they show considerable leaf necrosis in nursery or variety tests containing varieties susceptible to crown rust. In 1948 the leaf spots appeared before crown rust became

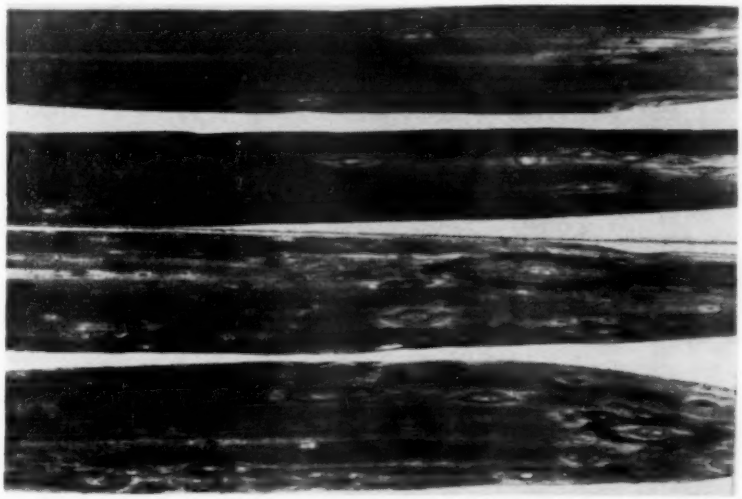

FIG. 1. Leaf spots on oats caused by *Helminthosporium victoriae.* Top, Victorgrain, greenhouse inoculation; bottom, DeSoto, natural infection.

prevalent, but in 1949 crown rust appeared ahead of the leaf spots. In 1950 the leaf spots were first observed on February 7. Varieties susceptible to Helminthosporium blight develop leaf spots several days or weeks prior to the appearance of dark or blackened nodes. However, the basal culm invasion probably causes more damage than the leaf spots.—Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

The Inactivation of the Carnation-Mosaic Virus.[1] GAIL E. RUMLEY AND W. D. THOMAS, JR. In attempting to select mosaic-free carnation plants for propagation, much infection has been encountered in nearly all commercial benches. Several viruses have been inactivated thermally *in vivo,*[2] and others have been inactivated chemically *in vivo*[3] and *in vitro.*[4]

[1] Published as Scientific Journal Series Article No. 332, with the approval of the Director of the Colorado Agricultural Experiment Station.

[2] Kunkel, L. O. Heat treatment for the cure of yellows and other virus diseases of peach. Phytopath. 26: 809–830. 1936.

[3] Stoddard, Ernest M. The X-disease of peach and its chemotherapy. Conn. Agr. Exp. Sta. Bul. 506. 1947.

[4] Takahashi, William N. The inhibition of virus increased by malachite green. Sci. 107: 266. 1948.

Jones[5] has reported that carnation mosaic may be inactivated at 60° C., but he did not indicate the time of exposure. Because of the common occurrence of mosaic in carnations an attempt was made to inactivate the virus as a means of controlling the spread of the disease.

Dianthus barbatus expresses unusually clear symptoms of mosaic;[6] therefore, it was used as the test plant for studies on thermal inactivation of mosaic virus. Inoculum was obtained by expressing juice from frozen, mosaic-infected carnations. This extract was centrifuged for 10 min. at 1700 r.p.m. and filtered through a celite filter. Three ml. of extract were used in each of six test tubes in the experiment, *in vitro*. Five of the tubes were subjected to temperatures of 40°, 50°, 60°, 70°, and 80° C., respectively, for 10 min. in a water bath. One tube was not subjected to heat. Following thermal treatment of the virus material, five healthy plants were inoculated with the heat-treated extract for each temperature exposure; five other healthy plants were inoculated with an untreated extract for a check. Inoculations were made by the abrasive technique, using No. 200 carborundum powder as the abrasive. Each plant was inoculated three times to insure better infection.

Exposures of extracts to temperatures between 40° and 80° C. for 10 min. resulted in a gradual decline in the virulence of the virus, the decline being most abrupt above 60° C. No infection occurred on plants inoculated with extracts which had been subjected to temperatures of 70° C. or above. All plants inoculated with the unheated extract manifested mosaic symptoms.

Attempts to inactivate the mosaic virus *in vivo* by maintaining infected plants at approximately 38° C. were unsuccessful, because of the extremely high mortality of the plants resulting from heat injury.

The nine chemicals used successfully by Stoddard were tested for their efficacy against carnation mosaic virus. Sulfathalidine, sulfathiazole, sulfamerizine, sulfaguanidine, and sulfasuxidine were used at a concentration of 0.0166 per cent, 8-hydroxyquinoline-sulfate and zinc sulfate at 0.025 per cent, hydroquinone, 0.0125 per cent, and calcium chloride, 0.2 per cent. Twelve unrooted, infected carnation cuttings were used for each test, together with 12 infected cuttings to be used as untreated checks. These cuttings were rooted in fine vermiculite in enameled pans 10 in. in diameter and 4 in. deep. The first watering of each lot consisted of 250 ml. of each chemical solution. Subsequent waterings were made with tap water. All rooted cuttings were potted in steam-sterilized soil 4 weeks after treatment. After 2 months they were tested for the presence of the virus by means of infection response to ultraviolet radiation[7] and by cross-inoculations from *D. caryophyllus* to *D. barbatus*.

[5] Jones, Leon K. Mosaic, streak, and yellows of carnation. Phytopath. 35: 37–46. 1945.

[6] Brierley, Philip, and Floyd F. Smith. Carnation and gladiolus virus diseases pose serious problems. Flor. Rev. 99 (2567): 30–33. 1947.

[7] Thomas, W. D., Jr., and August Mussenbrock. Selection of virus-free carnations by fluorescence. Flor. Rev. 102 (2648): 41. 1948.

Treatment of infected, unrooted carnation cuttings with the different chemicals indicated that calcium chloride and zinc sulfate may be effective against the mosaic virus *in vivo*. Half of the plants treated with zinc sulfate and calcium chloride recovered. Recovery was slight with 8-hydroxyquinoline sulfate; only one healthy plant was obtained. No mosaic resulted when extracts from the recovered plants were passed to healthy *D. barbatus*. Hydroquinone was toxic to unrooted cuttings; all but one died following treatment. The surviving cutting, however, was free from infection. Treatments with sulfathalidine, sulfathiazole, sulfamarizine, sulfaguanidine, and sulfasuxidine were toxic to the cuttings. Infection remained active in all untreated plants. Similar results were obtained upon repeating this experiment.

These experiments indicate that the carnation-mosaic virus may be inactivated chemically *in vivo*. However, the phytotoxic effect of the chemicals makes treatment of unrooted cuttings impractical.—Department of Botany and Plant Pathology, Colorado A & M College, Fort Collins, Colorado.